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## DETERMINATION OF ORGANIC ACIDS

### III. NOTE ON THE USE OF THE ISOAMYL ETHER-WATER SYSTEM IN THE PARTITION METHOD

C. H. WERKMAN

*From the Section of Bacteriology, Iowa Agricultural Experiment Station*

Accepted for publication May 20, 1930

The partition method for the quantitative determination of fatty acids in mixtures employs the differential distribution of the acids between either isopropyl ether and water (1) or ethyl ether and water (2). In the present paper necessary data are presented in tabular and graphical form for the use of the system of isoamyl ether and water. These data enable quantitative determinations to be made of the relative percentages of two fatty acids in a mixture. For a discussion of the principles underlying the partition method the reader is referred to previous papers (1 and 2). In the present paper only brief mention will be made of the details of procedure.

The *partition constant* is the number of cubic centimeters of 0.1 N alkali required to neutralize 25 cc. of the aqueous phase after 30 cc. of 0.1 N mixture of acids have been shaken with 20 cc. of the ether at 25°C.

The data for the use of the isoamyl ether-water system are presented because use of this system will be made in connection with the ethyl ether-water system in a method for the provisional identification of two fatty acids in a mixture. Although isoamyl ether would not ordinarily be used in routine determinations because of its relative cost, there is no reason, other than economy, for not doing so.

The mutual insolubility of isoamyl ether and water enhances its value and in this respect it is superior to the two ethers now employed in the partition method.

Isoamyl ether has a molecular weight of 158.2, a specific gravity of 0.774, and is insoluble in water but soluble in all proportions in ethyl alcohol or ethyl ether. It has a boiling point of 169-172° C. Upon vigorous shaking with water, it forms an emulsion which separates into insoluble phases slightly less quickly than the ethyl ether-water system.

The *partition constants* for the isoamyl ether-water system, given in table 1 were obtained at 25°C. by adjusting the original solution to 0.1N and partitioning the acids between 30 cc. of the 0.1N solution and 20 cc. of isoamyl ether. Twenty-five cc. of the aqueous phase were titrated with 0.1N alkali with phenolphthalein as the indicator. Graphs of the partition constants as abscissae against percentage composition of the mixture are shown in figure 1.

Formic acid behaves similar to acetic acid and can not be determined accurately by the partition method. Fermentations do not generally produce significant quantities of formic acid and small quantities present will be calculated as acetic. Fermentations generally result in the production



TABLE 1. *Experimental values of isoamyl ether-water partition constants. Twenty-five cc. aqueous phase at 25 C.*

Percentage 0.1N acid first named	Acetic-propionic	Acetic-butyric	Acetic-lactic	Propionic-butyric	Propionic-lactic	Percentage 0.1N acid second named
100	24.3	24.3	24.3	21.4	21.4	0
90	24.0	23.6		21.0		10
80	23.75	22.90		20.5		20
70	23.5	22.10		20.1		30
60	23.2	21.40		19.6		40
50	22.9	20.60		19.2		50
40	22.6	19.90		18.7		60
30	22.3	19.19		18.2		70
20	22.0	18.40		17.8		80
10	21.7	17.6		17.4		90
0	21.4	16.9	26.0	16.9	26.0	100

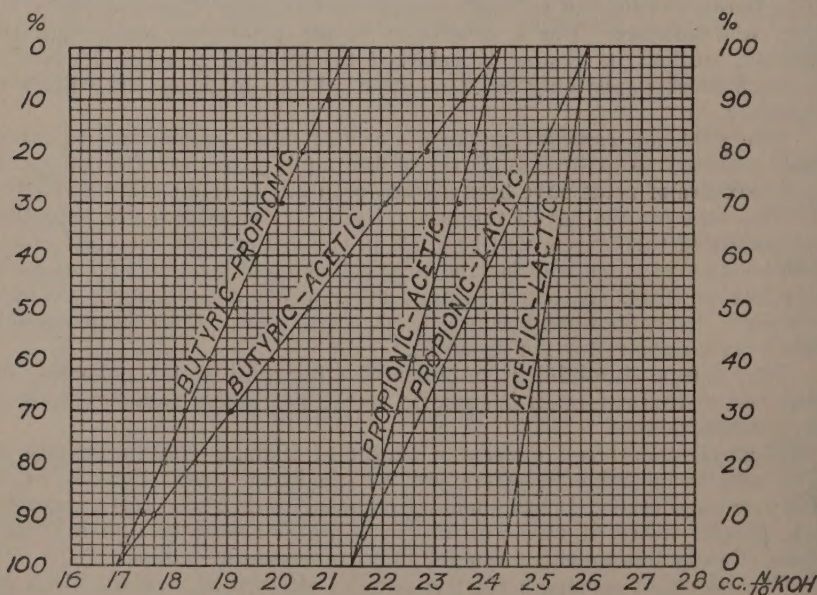


Fig. 1. Partition constants for the quantitative determination of two fatty acids distributed between isoamyl ether and water.

of substantial quantities of one or two volatile acids with small amounts of a third acid such as formic. The formic may be oxidized and thus determined if it is desired.

The general equations given in previous publications 1 and 2) for algebraic solution using the isopropyl ether and ethyl ether-water systems hold equally well for the use of isoamyl ether and will not be repeated here.

#### CONCLUSIONS

The partition method for the quantitative determination of two fatty acids in a mixture has been extended to include the use of the isoamyl ether-water system.



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## HYDRATION IN SWEET CORN

A. L. BAKKE AND A. T. ERWIN

*From the Sections of Botany and Plant Pathology, and Horticulture and Forestry,  
Iowa Agricultural Experiment Station*

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It is generally known that the seed of sweet corn often does not germinate as well as that of dent corn but the cause of this difference has not been determined. It seems, therefore, that a study of the intake of water under varying conditions might afford an explanation for the difference in germination of the two kinds of corn.

Babcock (2) found that when seed corn is placed in water, it will absorb more than its own weight in a few hours, and will increase considerably in volume. Duvel (6) stated that moisture is the prime factor causing the premature death of the seed.

Bailey (3) made a few preliminary tests upon the hygroscopicity of white dent corn and sweet corn. Fifty seeds of each were spread out on pieces of paraffined wire gauze which were suspended in the upper part of half-gallon museum jars, which contained different concentrations of sulfuric acid ranging from 30 to 85 per cent. The grain was exposed to the several atmospheres until no further change in weight took place. In all cases the dent corn absorbed a greater amount of moisture than the sweet corn.

Coleman and Fellows (4) studied the question of the hygroscopic moisture of cereal grains and flax, and demonstrated that absorption was greater for corn than for flax or wheat. Dillman (5) was interested in the hygroscopic moisture because of its bearing upon combine harvesting and subsequent storage. He pointed out that wheat, corn, buckwheat, and rice, possess about the same moisture content when equilibrium is reached.

### METHODS OF SECURING DIFFERENT HUMIDITIES

The sweet corn employed in these studies was the well known variety, Stowell's Evergreen. The Reid's and Hogue's yellow dent varieties were employed for comparative tests. These ears were as nearly as possible of the same maturity as the sweet corn. The ears of corn were picked directly from the field, to represent four stages: (1) milk, (2) early dough or canning, (3) early dent, (4) mature. These were separated into lots, I, II, III, and IV. The corn was stored in a dry ventilated room where the temperature was approximately 22°C. Germination tests of both sweet and field corn showed that all seeds were viable. Before being used, all seeds were treated with hypochloride of lime.

To secure humidities of varying degrees, the sulfuric acid method of Wilson (8) was used. With a specific gravity of 1.05 there is 7.37 per cent acid, and a relative humidity of 97.5 per cent. For a humidity of 80.5 per cent, the amount of sulfuric acid is 27.32 per cent and the resulting specific gravity becomes 1.20. For a humidity of 60.7 per cent the concentration of sulfuric acid is 38.03 per cent, while the specific gravity has a value



of 1.29. The 100 per cent relative humidity was, of course, obtained by using distilled water alone. All of the hygroscopic tests were carried on in sealed containers. The sulfuric acid was held in a crystallizing dish resting upon the glass plate. Over the container was inserted an open topped bell jar. The rubber stopper of the bell jar served as a means by which the wire, holding the shallow screen tray with the seeds, could be suspended directly over the crystallizing dish 4 cms. from the water surface. A covering of brown paper excluded the light.

#### IMBIBITION OF SWEET CORN AND FIELD CORN

The purpose of the first series of experiments was to determine the extent or maximum absorption for sweet corn when left in distilled water. Ten-gram samples of sweet corn having a 9.1 per cent moisture content were placed in distilled water where they were allowed to remain for 72 hours: during this time they were weighed at frequent intervals. At the time of each weighing the water was poured off, and the adhering water absorbed with filter paper. The seeds were then returned to the original container and water added. The data secured are shown in table 1.

TABLE I. *Absorption in sweet corn seed submerged in distilled water*

Hrs. submerged	7	9	12	20	23	29	33	48	60	72
Percentage gain	29.6	32.9	38.8	53.2	57.1	62.7	66.3	76.7	87.1	96.9

At the end of 7 hours the average absorption was 29.6 per cent and after 72 hours 96.9 per cent. The rapid rate of absorption shortly after the seeds were placed in the water was particularly noticeable. It is clear that data should have been collected earlier.

The next series of experiments was planned so that particular consideration could be given to the extent of absorption for shorter periods of subjection than seven hours. Weighings were made at the end of 1, 2, and 4 hours, and extended over a longer period in order to be sure that maximum absorption had been reached. For a comparison, Hogue's yellow dent corn was used. The data are given in table 2.

The data given in table 2 and the graph (Fig. 1) show that when sweet corn and field corn are placed in distilled water, the sweet corn absorbs a greater amount than dent corn. The equilibrium point for both the sweet corn and the dent corn is reached at about the same time. However, the sweet corn seed absorbs water to the extent of 113.37 per cent, while for dent corn the intake does not exceed 73.7 per cent.

#### THE RATE AND EXTENT OF ABSORPTION OF CORN SEED

It is desirable to determine the effect which various external factors may have upon the seed with varying moisture contents. An attempt was made to determine the rate and amount of absorption. The sweet corn used in these trials was the same variety but was different in moisture content; the Reid yellow dent has a water holding content of 8 per cent. The rate of absorption in distilled water is shown in table 3.

TABLE 2. *Percentage absorption occurring in Stowell's ever-green sweet corn and Hogue's yellow dent corn in distilled water for periods from one to 241 hours*

Time hours	Sweet corn	Dent corn
	Percentage gain	Percentage gain
1	11.85	8.87
2	17.37	13.97
4	26.15	20.30
7	33.35	25.55
9	39.34	29.20
11	43.07	32.25
13	46.97	34.25
15	49.30	38.42
20	55.22	39.85
22	58.82	41.27
24	61.40	42.99
28	65.15	43.07
32	67.52	46.77
41	74.92	50.67
45	78.20	51.27
50	80.25	53.37
54	82.22	54.62
62	86.82	57.97
74	92.40	60.42
87	97.45	64.87
100	101.72	66.55
112	105.15	69.10
124	107.86	70.35
148	110.75	72.12
172	113.32	73.70
241	113.37	70.45

TABLE 3. *Percentage increase in weight of sweet corn and dent corn in distilled water at 25°C.*

Sweet corn		Dent corn	
Percentage increase	Time-hours	Percentage increase	Time-hours
12 to 17	1 to 2	12 to 14	1 to 2
17 - 26	2 - 4	14 - 20	2 - 4
25 - 35	4 - 7	20 - 25	4 - 7
35 - 40	7 - 9	25 - 30	7 - 9
40 - 45	9 - 12	30 - 35	9 - 11
45 - 50	12 - 15	35 - 40	14 - 22
50 - 60	15 - 24	40 - 45	22 - 28
60 - 70	24 - 32	45 - 50	28 - 42
70 - 80	32 - 50	50 - 55	42 - 55
80 - 90	50 - 74	55 - 60	55 - 75
90 - 100	74 - 100	60 - 65	75 - 88
100 - 110	100 - 148	65 - 70	88 - 124
110 - 115	148 - 175	70 - 75	124 - 173

In the data given, the moisture content of the sweet corn having an initial value of 10 per cent produces an imbibitional increase of 40 per cent in nine and 22 hours respectively. At the end of 15 hours the sweet corn has taken up a sufficient amount of water to bring the increase in weight of the sweet corn up to 50 per cent, while the dent corn does not give this result until after a lapse of 28 hours. To produce an imbibition of 60 per cent the time factor was 24 hours for the first named strain and 55 hours for the second, while for 70 per cent, the respective subjection periods were respectively 32 and 124 hours. The maximum hydration was found to be approximately the same in both cases.

#### HYGROSCOPICITY OF SWEET CORN AND DENT CORN

Sweet corn and dent corn are often stored where the humidities are high. The next series of absorption tests was made to determine the differences in hygroscopicity between sweet corn and dent corn. The humidities arbitrarily chosen were 100, 95 and 90 per cent. The seed used in these tests was harvested at different stages of maturity and divided into four different lots: I Milk, II Canning, III Early dent, and IV Mature. The corn was dried and stored under identical conditions. The results secured in a saturated atmosphere are in table 4 and shown graphically in figure 2.

TABLE 4. *Absorption of moisture by sweet corn and dent corn in an atmosphere of 100 per cent humidity at 22°C.*

Lot	Variety of corn	Time in hours						
		20	44	68	94	116	140	168
I	Sweet	22.18	32.34	35.77	38.25	41.25	44.34	46.17
	Dent	17.65	27.82	32.60	34.15	37.99	42.37	43.97
II	Sweet	20.38	29.01	33.62	33.41	39.66	43.29	45.67
	Dent	15.41	24.17	32.92	-----	33.36	36.33	38.38
III	Sweet	15.98	26.38	34.36	41.08	47.08	51.73	54.78
	Dent	15.59	27.56	33.16	38.35	43.72	50.45	51.96
IV	Sweet	18.71	28.08	36.41	41.72	46.71	52.87	55.65
	Dent	18.74	33.22	42.01	46.55	51.34	56.49	48.41
Average	Sweet	19.31	28.95	35.04	38.61	43.67	48.05	50.56
	Dent	16.84	28.19	35.17	29.74	41.72	46.41	45.68

Examining the data as submitted in table 4, it appears that lot I throughout the test shows a greater hygroscopicity for the sweet corn than for the dent corn; the same is true for lot II. In lot III the dent corn gives a higher reading. In lot IV the sweet corn has the lower value up to the 140 hour reading. Considering the average data the sweet corn is higher than the dent corn except on the 68 hour reading. When absorption by the less mature corn is compared with that of the more mature, there seems to be indications that the higher hygroscopicity is associated with maturity.

The next series of tests of hygroscopicity of corn was concerned with the extent of absorption in an atmosphere where the humidity was 95 per cent. The data secured are presented in table 5.



TABLE 5. *Rate of absorption of moisture by sweet corn and dent corn in an atmosphere of 95 per cent humidity at 22°C.*

Lot	Variety of corn	Time in Hours					
		20	68	94	116	140	168
I	Sweet	16.36	15.40	30.44	22.90	25.02	28.05
	Dent	14.23	15.73	23.14	19.93	26.99	23.06
II	Sweet	16.04	14.82	27.08	22.10	28.41	23.63
	Dent	12.20	14.59	22.20	18.27	25.10	24.32
III	Sweet	13.45	16.27	25.12	25.43	33.22	34.93
	Dent	12.74	15.39	24.23	22.78	32.02	30.31
IV	Sweet	16.20	19.85	27.33	26.32	34.62	31.14
	Dent	14.81	15.81	26.56	25.49	31.05	31.97
Average	Sweet	15.51	16.58	27.49	24.18	30.06	29.18
	Dent	13.49	15.38	24.03	21.61	28.79	27.41

It is apparent that the resistance to absorption has been increased, for the maximum moisture intake was never above 30 per cent, while in the 100 per cent chamber the increase became almost twice as much. For the first 68 hours there is generally greater absorption registered for the sweet corn. Some time after an exposure of 68 hours there is a decided drop in the extent of the intake, for the 94 hour readings give a decrease, while 22 hours later there is a decided increase over the earlier readings. This apparent discrepancy may possibly be attributed to a drop in the room temperature. Maximum absorption is reached after an exposure of 116 hours; after that there is a decrease. This may be explained as being a result of germination.

The next series of hygroscopicity tests was made where the humidity was adjusted to 90 per cent. The method of procedure was the same as before. The data are presented in table 6.

TABLE 6. *Rate of absorption of moisture by sweet corn and dent corn in an atmosphere of 90 per cent humidity at 22° C.*

Lot	Variety of corn	Time in hours						
		20	44	68	94	116	140	168
I	Sweet	13.47	16.21	22.15	17.85	23.09	19.15	17.20
	Dent	13.02	14.45	17.89	18.38	25.75	20.48	18.16
II	Sweet	22.81	16.85	25.82	19.50	25.41	18.95	18.03
	Dent	13.69	14.68	21.76	18.27	23.70	18.75	18.09
III	Sweet	12.63	14.90	24.98	26.36	25.50	19.65	16.66
	Dent	13.21	15.33	24.71	26.42	34.14	29.50	26.30
IV	Sweet	20.80	18.12	28.76	24.30	30.97	26.55	22.91
	Dent	20.51	16.35	26.39	22.40	31.54	25.60	22.11
Average	Sweet	19.92	26.52	25.42	19.50	26.24	21.07	18.70
	Dent	15.10	15.20	22.68	21.36	28.78	23.58	23.66

The results do not show correspondingly lower values between the 95 and 90 per cent humidity as noted between the 100 per cent and 95 per cent. With the lower humidity it is to be expected that the absorption would be somewhat less. This did not always prove to be the case. For the first 68 hours, however, the readings are consistently lower than those for the 95 per cent humidity, indicating an increased resistance to absorption. Lot IV (mature) for the first 116 hours shows a greater increase. It is noted also that there are lower values registered for 116 hours than for 94 hours or for 140 hours. The reason for this is probably the same as suggested for the lower readings at the 94th hour in the preceding experiment, namely a lowered temperature. It is noticed too, that the readings taken at the end of 140 hours are higher. The lower values recorded for the last readings are, with one exception, lower. Whether this can be attributed to increased respiration cannot be definitely stated.

In the data submitted previously there are indications that mature corn placed or held in an environment where the humidity is high will absorb a greater amount of moisture in a given time than cured and dry green corn kept in the same place. The data are given in table 7.

TABLE 7. *Percentage gain in sweet corn and dent corn of varying stages of maturity when subjected to a humidity of 100 per cent for varying periods from 20 to 168 hours*

Lot	Variety of corn	Time in hours						
		20	44	68	94	116	140	168
I	Sweet	19.02	26.00	27.47	30.28	31.05	33.10	34.61
	Dent	16.98	23.16	21.86	27.25	30.32	33.58	32.26
II	Sweet	17.08	22.94	27.90	27.35	30.22	32.37	32.42
	Dent	16.11	22.06	24.96	24.36	28.11	30.40	31.60
III	Sweet	16.35	23.82	27.33	31.86	36.65	40.88	42.38
	Dent	15.31	30.10	25.41	31.80	36.07	40.39	41.23
IV	Sweet	24.77	21.94	29.68	34.29	38.48	43.41	44.45
	Dent	20.30	38.87	32.59	35.60	40.81	44.08	43.33

The data as given in table 8 point toward a gradual increase in hydration capacity with maturity. It is true that there is little difference in the results submitted for lots I and II. This is not unexpected, for the time of transition is exceedingly short and somewhat indefinitely marked. (1). The corn selected from lots III shows a much higher hygroscopicity and the same is true for the corn of lot IV. The differences in hygroscopicity represented by sweet corn and dent corn for lots or stages I, II, and III are shown in graphs in figures 3, 4 and 5. Probably the increased hydration of mature sweet corn and dent corn over the immature is caused by the production of pentosans (7), and other materials which increase the capacity for water intake.

If corn has a lower hygroscopicity when harvested and cured during the early dented stage, it is logical to advocate the harvesting of seed corn at such a time.

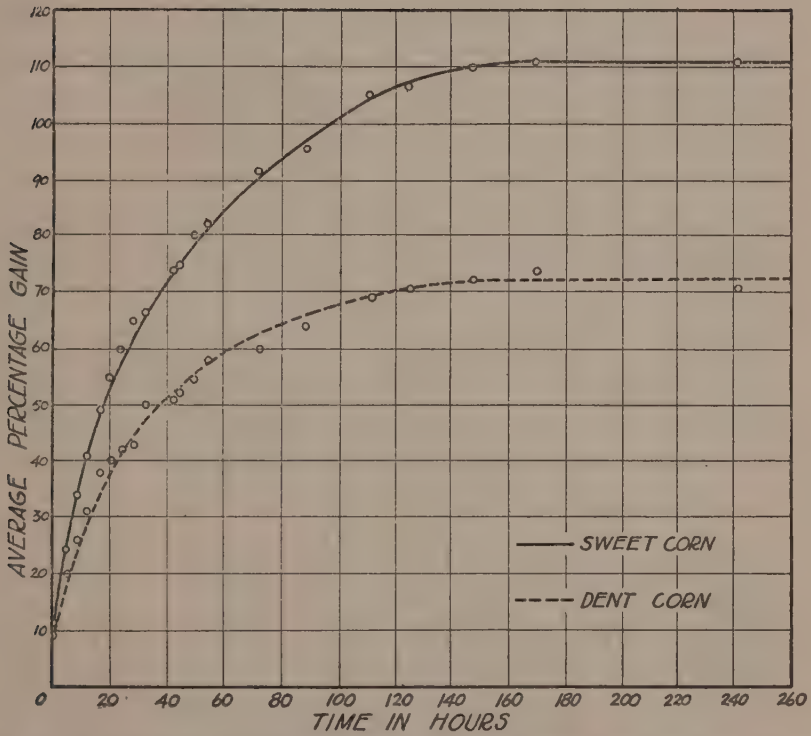


Fig. 1. Comparative rate of absorption of moisture in sweet and field corn. Note that the sweet corn absorbs considerably more moisture than the field corn in the same period of time.

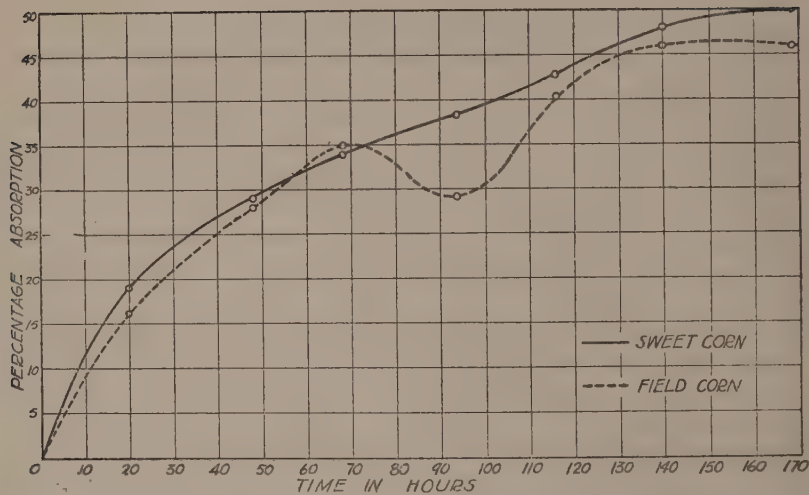


Fig. 2. Comparative rate of absorption of moisture of sweet and field corn in a saturated atmosphere at a temperature of 22°C.

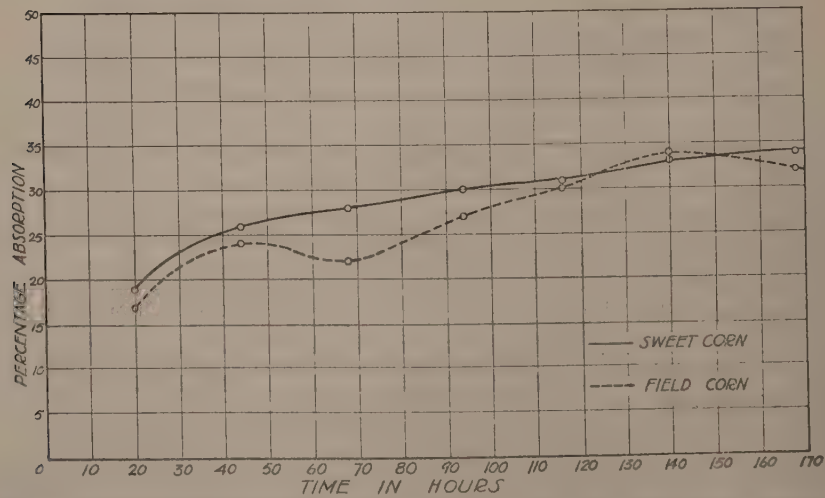


Fig. 3. Comparative rate of absorption in sweet and field corn which were picked in the milk stage, dried and then subjected to a saturated atmosphere.



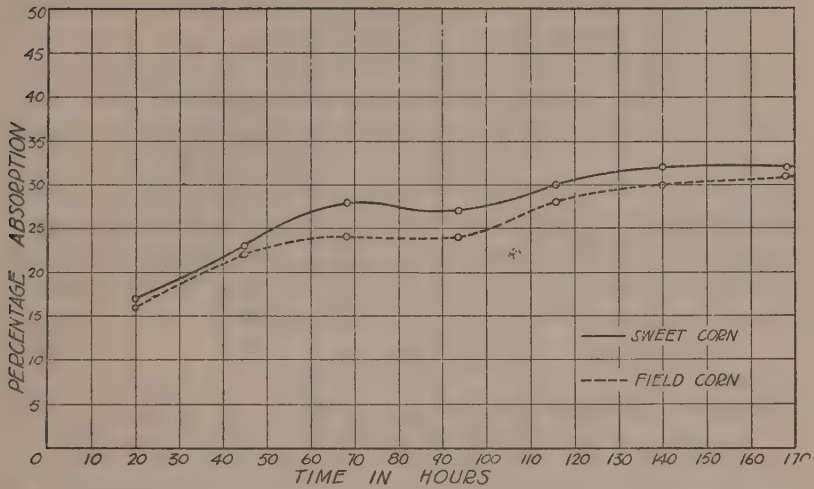


Fig. 4. Comparative rate of absorption in sweet and field corn which were picked in the canning stage, dried and then subjected to a saturated atmosphere.

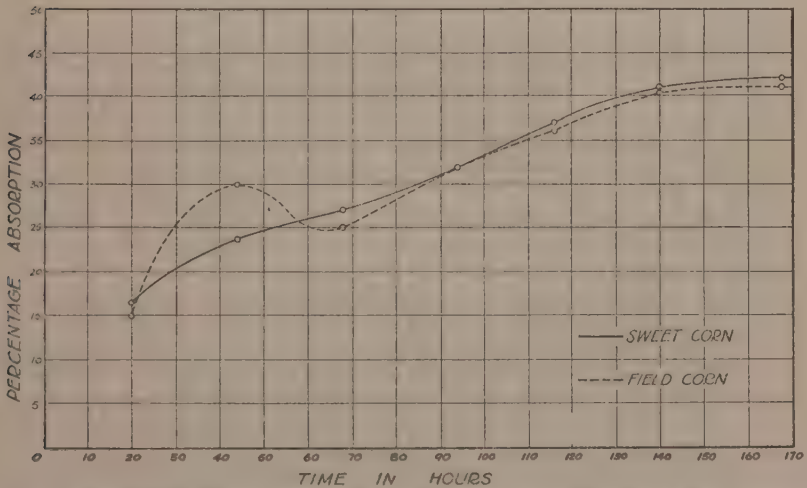


Fig. 5. Comparative rate of absorption in sweet and field corn which were picked in the early dent stage, dried, and then subjected to a saturated atmosphere.

## SUMMARY

Cured sweet corn seed when placed in distilled water increases its weight 113.37 per cent; dent corn 73.7 per cent.

The rate of absorption is greater in sweet corn than in dent corn. Sweet corn placed in distilled water increases 50 per cent in 15 hours while the rate for a similar increase in dent corn is 28 hours.

Both sweet and dent corn absorb less moisture when there is a decrease in the humidity. In an atmosphere of 100 per cent humidity the maximum hygroscopicity of sweet corn is 50.56 per cent; dent corn, 45.68 per cent. At 90 per cent the respective values are 30.06 and 28.79 per cent.

The hydration capacity of cured sweet corn increases with maturity, passing from 32.42 per cent in the milk stage to 42.38 per cent for the early dent and 44.45 per cent when harvested mature. For dent corn the values are respectively 31.60, 41.23 and 43.33 per cent.

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# THE CANNIZZARO REACTION WITH FURFURAL<sup>1</sup>

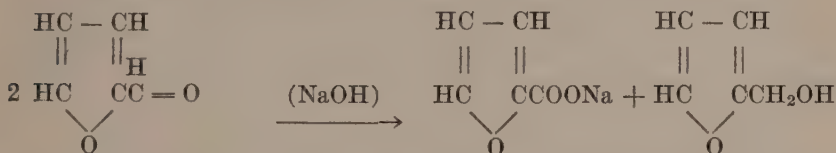
HENRY GILMAN AND WILLIAM M. SELEY

*From the Chemical Laboratory of Iowa State College*

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## INTRODUCTION

The Cannizzaro reaction is one of the best methods for the preparation of acids and alcohols from aldehydes. It has been extensively used for the synthesis of pyromucic acid and furfuryl alcohol from furfural<sup>2</sup>.



The need for improvements in this reaction has become urgent in view of the increasing number of substituted furfurals developed in this laboratory<sup>3</sup>.

As a result of a miscellany of studies reported at this time, it is now possible to carry out the Cannizzaro reaction on furfural with greater convenience and improvements in yield. The method, which involves the use of an alcoholic solution of sodium hydroxide, is recommended particularly for substituted furfurals which do not contain a substituent sensitive to alcoholic-alkali solutions. It should also prove suitable for small-scale laboratory condensations involving furfural. Actually, the method has been tried with success as a student laboratory exercise in an undergraduate course in organic chemistry<sup>4</sup>.

Hill<sup>5</sup> used alcoholic-sodium hydroxide and Hartley and Dobbie<sup>6</sup> used alcoholic-potassium hydroxide to prepare pure pyromucic acid from fur-

<sup>1</sup>This is one of a series of studies in organic chemistry concerned with the utilization of agricultural wastes. The authors gratefully acknowledge help from the Industrial Science Research Fund for the partial defrayal of expenses incurred in this investigation.

<sup>2</sup>Leading references to this reaction may be found in Bulletin No. 2, June, 1928, entitled "Furfural and Its Derivatives," published by the Miner Laboratories, Chicago, Ill. See, also, *Organic Syntheses*, 6, 44-47 (1926).

<sup>3</sup>See, Gilman and Wright, *J. Am. Chem. Soc.*, 52, 1170, 2550 (1930), for the preparation of bromofurfural and nitrofurfural. Since then other new substituted furfurals have been synthesized in this laboratory.

<sup>4</sup>Recently Gilman, Brown, Wright and Hewlett, *Iowa State College Jour. Sci.* 4, 355 (1930), directed attention to the Perkin reaction with furfural (leading to the preparation of furylacrylic acid) as another student preparation. Such preparations are admirably suited for general laboratory instruction in organic chemistry, and have the special merit of starting with the very inexpensive furfural.

<sup>5</sup>Hill, *Am. Chem. J.*, 3, 33 (1887).

<sup>6</sup>Hartley and Dobbie, *J. Chem. Soc.*, 73, 598 (1898).

fural. They made no study of optimal conditions, did not isolate the furfuryl alcohol, and did not report yields of the pyromucic acid. Their alkali was dissolved in ethyl alcohol. In our experiments, only methyl alcohol was used, partly because we were interested in a preparation for undergraduates which would not involve the use of ethyl alcohol. For technical purposes, the use of our sodium hydroxide is, of course, to be preferred to potassium hydroxide.

Studies are in progress on the interconversion of the so-called water soluble and water insoluble forms of furfuryl alcohol. If it should prove possible to readily effect such interconversion then the isolation of alcohol from the Cannizzaro reaction should be markedly simplified.

#### EXPERIMENTAL PART

Three different methods were used for the extraction of the furfuryl alcohol. First, the reaction mixture was extracted in a separatory funnel; second, extraction was effected in a Soxhlet apparatus; and, third, the sodium pyromucate contained on a Büchner funnel was washed with the extracting solvent.

The furfural was redistilled material, and the sodium hydroxide solution was prepared by dissolving 20 g. (0.5 mole) of sodium hydroxide in 145 cc. of methyl alcohol. Such solution was accelerated by stirring or shaking.

In the first series of experiments (extraction in a separatory funnel), 96 g. (1.0 mole) of furfural was mixed with the solution containing 20 g. (0.5 mole) of sodium hydroxide in 145 cc. of methyl alcohol. The reaction was carried out in a Erlenmeyer flask. A yellow mass precipitated in a very short time and set to a solid with a reddish tint. The reaction is distinctly exothermic, and for this reason cooling was effected by tap water. After standing (generally for twelve hours) the mixture was dissolved in water and extracted with ether until the extracts were only slightly red in color. The ether extracts were distilled at atmospheric pressure and that portion distilling between 167°-170° was collected separately. This was the furfuryl alcohol, and its pale yellow color changed on standing to a reddish color<sup>7</sup>. The solution containing sodium pyromucate was heated on a water-bath to expel residual ether, and was then acidified with 50 per cent sulfuric acid. In this manner, the yellowish crystals of pyromucic acid (melting at 127°) were precipitated. Experimental details of this method are contained in table 1, which describes a series of preparations starting with one mole of furfural and one-half mole of sodium hydroxide.

Two experiments were carried out using a Soxhlet extractor. Each mixture resulting from 0.5 mole of furfural and 0.25 mole of sodium hydroxide was allowed to stand 12 hours prior to transferral to a Soxhlet extractor. Extraction with 250 cc. of ether was continued until the ether washings were clear. The extracts were distilled in the customary manner, again collecting the furfuryl alcohol between 167°-170°. The sodium salt

<sup>7</sup>The use of a very small amount of urea has been recommended as a stabilizer for furfuryl alcohol. (See, *Organic Syntheses*, 6, 46 (1926).



TABLE I. *Extraction of furfuryl alcohol in separatory funnel*

Solvent used in extraction	Vol. of solvent	Pctg. yield of acid	Pctg. yield of alcohol
Ether	800 cc.	58.0	87.0 <sup>(a)</sup>
Ether	800 cc.	30.0	33.6 <sup>(b)</sup>
Ether	200 cc.	25.0	27.0 <sup>(c)</sup>
Benzene	800 cc.	56.0	42.0 <sup>(a)</sup>
Benzene	200 cc.	20.0	35.0 <sup>(d)</sup>
Carbon Tetrachloride	200 cc.	.....	10.8 <sup>(e)</sup>

<sup>(a)</sup> The condensation mixture was allowed to stand at room temperature 12 hours before being worked up. It remains for further experiments to determine whether this time for reaction can be materially decreased.

<sup>(b)</sup> In this experiment the mixture was kept below 50°, and the time of standing was 5 hours.

<sup>(c)</sup> Very probably the 200 cc. of ether used for extraction in this experiment was inadequate, because on acidification of the sodium salt a tarry mass developed. This indicates the presence of unextracted furfuryl alcohol because the pyromucic acid is stable towards the inorganic hydrolyzing acid under these conditions.

<sup>(d)</sup> Here, also, the formation of an apparently intractable tarry mass on acidification indicated that not all of the furfuryl alcohol had been extracted.

<sup>(e)</sup> The acid was not recovered from the tarry mixture resulting on acidification.

Attention should be directed to a single experiment where extraction was replaced by steam distillation. Only a small quantity of furfuryl alcohol was obtained in the distillate, and when the residue from steam distillation was acidified a thick, gummy mass formed, and this yielded 18 per cent of acid.

was acidified with 50 per cent sulfuric acid, and the acid obtained in this manner melted at 127°. In an 8 hour period of extraction, the yield of acid was 70 per cent and the yield of alcohol, 50 per cent. In a corresponding experiment where the time of extraction was but one hour, the respective yields were essentially the same.

In the third group of experiments, the reaction mixture (after standing for 12 hours) was transferred to a Büchner funnel and there washed with ether until the washings were essentially free of color. The furfuryl alcohol and pyromucic acid were then worked up in accordance with the procedures described for the other two methods. The boiling point and melting point, respectively, of the two products were alike in all three methods. Two experiments were carried out under these conditions with 1.0 mole of furfural and 0.5 mole of sodium hydroxide. The yields were: 50 per cent and 75 per cent, respectively, of acid; and 56 per cent and 34 per cent, respectively, of alcohol. In that experiment giving a 50 per cent yield of acid, some of the sodium salt of the acid was lost. Also, two experiments were carried out using 3.0 moles of furfural and 1.5 moles of sodium hydroxide. Here, the yields of acid were 71 per cent and 75 per cent, respectively; and the yields of alcohol, 41 per cent and 49 per cent, respectively. In these 3.0 mole runs it is necessary to cool the mixture in an ice-water bath to about 0°, otherwise a too vigorous reaction occurs.

It is evident that each of the three methods described has special advantages. The choice of a particular method for extracting the furfuryl alcohol depends somewhat on the size of a preparation and the availability of the substituted furfural used. Obviously, with a rare substituted-furfural it would be desirable to use that method which gives the highest yields of acid and of alcohol. It should be remembered that a large part of the solvent used for extraction can be recovered by usual procedures.

The authors gratefully acknowledge assistance from Mr. George F. Wright.

#### SUMMARY

Conditions have been described for effecting a Cannizzaro reaction with furfural and sodium hydroxide dissolved in methyl alcohol. The method is recommended particularly for substituted furfurals because of the improved yields and general convenience over other related procedures.

# A POLYMERIC 2-FURFURYL MERCAPTAN<sup>1</sup>

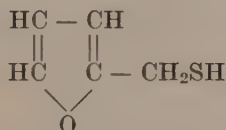
HENRY GILMAN AND A. P. HEWLETT

*From the Chemical Laboratory of Iowa State College*

Accepted for publication July 15, 1930

## INTRODUCTION

In connection with studies<sup>2</sup> concerned with applications of 2-furfuryl mercaptan, a compound obtained directly from furfural, it was noted that the exceedingly offensive smelling liquid mercaptan underwent a change on standing in a sealed tube to give an odorless solid. This metamorphosis is apparently not described in the literature. In order to elucidate the structure of the solid, it was distilled under reduced pressure and a chief component of such distillation was the liquid mercaptan. The solid and the liquid have the same percentage of sulphur and this with the inter-conversion of one form to the other is evidence that the solid is a polymer of the liquid 2-furfuryl mercaptan, the structural formula of which is



In this polymeric modification of 2-furfuryl mercaptan, we have one of the most striking illustrations of an all too frequently occurring phenomenon of furan compounds: namely, their tendency to ready, and in some cases complete, conversion to oils, tars and resins. The formation of such apparently intractable materials is the *bête noire* of furfural chemistry, because of the depressing ease with which many furan compounds assume almost intractable modifications. At the same time we should hasten to add that this same tendency to resin formation is, of course, of fundamental importance in the technical preparation of resins, plastics, and lacquers, from furfural and its derivatives.

It is well-known that there exists wide differences in the so-called stabilities of furan types. Some, like the acids, are uncommonly stable; others, like furan itself, amino furans and furan alcohols, are very unstable. Also, recent studies<sup>3</sup> have demonstrated that it is possible to pronouncedly stabilize otherwise labile molecules by the introduction of vari-

<sup>1</sup>This is one of a series of studies in organic chemistry concerned with the utilization of agricultural wastes. The authors gratefully acknowledge assistance from the Industrial Science Research Fund for the partial defrayal of expenses incurred in this investigation.

<sup>2</sup>Gilman and Hewlett, *J. Am. Chem. Soc.*, **52**, 2141 (1930).

<sup>3</sup>A series of such studies has been reported from the laboratories of organic chemistry in the last few years in the *Iowa State College Jour. Sci.*, in the *J. Am. Chem. Soc.*, and in the *Rec. trav. chim.*



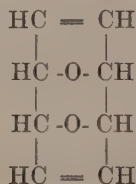


In addition to the highly reactive conjugated systems just mentioned there are the simpler units of unsaturation: 2, 3, and 4, 5, in addition to the nuclear oxygen and the extra-nuclear carbonyl group 6, 7.

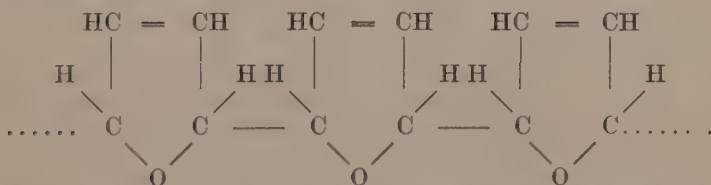
Finally, there is another possible highly reactive group which may participate in polymerization, and that is the enol (or the di-enol, in some cases) resulting from ring scission. It appears that the furan ring can be opened with relative ease by a number of reagents, and it is definitely known that the enol grouping is one of the most active organic linkages.

We have then at least three distinct types of linkages, real and potential, in furan compounds, any one or any combination of which might be responsible for the union of two or more molecules to give polymers. For want of any definite experimental evidence at this time, it is not possible to designate any one or any combination of such linkages which participate in all polymerizations. When polymerization involves extra-nuclear linkages, like the carbonyl group in furfural, then we have a peculiar system for each furan compound. The same is true, but to a lesser extent, in those cases where only the furan ring or better stated where essentially the furan nucleus takes part. In this latter case it is now possible to have a picture of the configuration or of the mode of linking of some polymers.

If we confine attention, for purposes of illustration, to the 2, 3, 4, 5, conjugated system<sup>6</sup>, then by 1, 4-addition of two molecules of furan itself we would have the following polynuclear heterocyclic type



If, however, we use the same compound and the same conjugated system, but have the polymerization extend beyond two units, then the following is a picture of such polymers:



The partial or latent or unsatisfied linkages designated by dotted lines can then be starting points for the addition of further units or for completing a system intramolecularly<sup>7</sup>.

<sup>6</sup>This system is selected because it is one of the chief systems designated by Gilman and Wright, *J. Am. Chem. Soc.*, 52, 3349 (1930), in an explanation of some substitution reactions of furfural.

<sup>7</sup>It should be noted that in these particular forms that a new unit of activity has been developed: namely, the unsaturation between elements 3 and 4. Should this unit prove to be relatively more reactive than the others in the designated molecules then it might prove possible to synthesize furan types with substituents on elements 3 and 4. Such elements otherwise greatly resist replacement of their hydrogen by substituents.

It should be possible to determine the correctness of the polymers indicated (as well as the other types which may be constructed from the other linkages mentioned) by the physical and chemical methods now available and in the process of being developed for other studies on polymerization. The problem is one of fundamental importance in extending our knowledge of furan compounds, particularly because of the apparent ease with which so many furan types polymerize<sup>5</sup>.

In some cases (see footnote 7) it may prove highly desirable to effect such polymerization and then depolymerize the molecule. This brings to mind the so-called water-insoluble form of furfuryl alcohol,  $C_4H_3OCH_2OH$ . The structure of this form is unknown. It may or may not be a polymer of the simple furfuryl alcohol, that remains to be determined from some studies now in progress. It is significant that the furfuryl alcohol is a simple analogue of thio-furfuryl alcohol or 2-furfuryl mercaptan. Should the water-insoluble form of furfuryl alcohol prove to be a polymer which would be readily converted to the water-soluble furfuryl alcohol, then it would be a ready means of decidedly improving the yield of furfuryl alcohol in the Cannizzaro reaction<sup>8</sup>.

#### EXPERIMENTAL PART

The 2-furfuryl mercaptan<sup>2</sup> was sealed in a glass tube and allowed to stand for one year. At the end of this time, the contents had become highly viscous and a white solid was suspended in the mixture. Filtration by suction served to remove the viscous oil from the solid. The oil had the highly characteristic odor of furfuryl mercaptan, but resisted steam distillation and underwent complete decomposition upon attempted distillation under reduced pressure.

The solid was thoroughly washed with anhydrous ether and then crystallized from ethyl acetate to yield an apparently pure compound, without odor, and melting sharply at 135°.

*Analysis* Calc. for  $(C_5H_6OS)_n$  : S, 28.07%. Found: S, 27.98%.

The value of  $n$  has not as yet been determined with definiteness, but it may be as high as 7 by boiling point determinations in ethyl acetate<sup>9</sup>.

Three grams of the polymer were heated at 10 mm., the temperature of the oil-bath being gradually increased to 150°. The distillate consisted of one gram (a 33.3% yield based on a polymer) of furfuryl mercaptan distilling at 155° at atmospheric pressure. The residue from the first distillation appeared to consist of a carbonaceous decomposition product. The furfuryl mercaptan was further characterized by the preparation of the *p*-nitrobenzoate. The identity of the *p*-nitrobenzoate was confirmed by a mixed melting point determination with an authentic specimen.

In a miscellany of studies by J. B. Dickey, G. F. Wright, R. E. Brown, E. A. Zoellner, S. A. Harris, W. M. Selby, N. O. Calloway and others in this laboratory it has been observed that furan compounds, as might have

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<sup>8</sup>See Gilman and Selby, *Iowa State College Jour. Sci.* (1930), for the Cannizzaro reaction. One of the major difficulties in this excellent synthesis is the removal or recovery of furfuryl alcohol.

<sup>9</sup>It is interesting to recall that molecular weight determinations of furan compounds indicate a general tendency to association.



been expected, show a greater tendency to remain pure when first purified with care and then carefully sealed<sup>10</sup>. With some liquids it appears desirable to effect such purification by distillation *in vacuo* rather than at atmospheric pressure.

A number of compounds which have been kept for varying times appear to be undergoing transformations which might be like that of furfuryl mercaptan. An interesting illustration is the simple ethyl furoate which is gradually being converted to a higher boiling compound.

It is noteworthy that some of the pure, freshly distilled furan compounds only develop their highly characteristic odors (and these are very pleasant with some esters, etc.) about one-half to one hour subsequent to distillation.

A study now in progress on "stabilizers" for furan compounds should make it possible to extend the utility of furan types.

#### SUMMARY

It has been shown that the liquid 2-furfuryl mercaptan polymerizes spontaneously to a solid, and that the polymer so formed can be converted by heating to the liquid. The solid form has none of the extremely obnoxious odor so characteristic of the liquid. In this preliminary study a series of mechanisms has been postulated for polymerizations of furan types, and some applications of the polymers have been considered.

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<sup>10</sup>There are some cases where it appears to be of advantage not to seal the compounds in order to permit volatile products of decomposition to escape and so diminish auto-catalytic decomposition or alteration.

It is interesting to note that furfural, quite unlike the analogous benzaldehyde, greatly resists atmospheric oxidation to furoic acid under conditions where benzaldehyde is readily converted to benzoic acid. However, preliminary experiments on the effect of ultra violet on furan compounds indicates a reaction between furfural and air at room temperature and in sealed containers, as a result of which the volume of confined air is reduced.



# THE VARIETAL RESPONSE AND INHERITANCE OF RESISTANCE IN BARLEY TO ERYSIPIHE GRAMINIS HORDEI P. F. 4

S. M. DIETZ<sup>1</sup>

From the Department of Botany, Iowa State College

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*Erysiphe graminis hordei* Marchal (powdery mildew) is a destructive parasite in most sections of the United States where barley is grown. Observations have shown that the conditions which favor the development of this parasite are cool temperature and humid atmosphere. However, the losses are particularly severe in the southern United States where barley is planted in the fall. The eastern half of the Mississippi Valley and coastal states in the western United States suffer an annual loss. In Iowa the barley has been relatively free from powdery mildew during the past twelve years, while in the neighboring states it has been severely attacked. The reason for this is not known.

At present there is available only limited information of the varietal response and the inheritance of resistance to *E. graminis hordei*. In 1907 Biffen<sup>2</sup> showed that resistance to *E. graminis hordei* was an inheritable character in crosses of *Hordeum spontaneum* (resistant) on *H. hexastichofurcatum* (susceptible). It is improbable, however, that he worked with a single physiologic form, because his experiments were carried out under field conditions on mature plants. The F<sub>2</sub> segregated into 56 susceptible, 16 intermediate, to 7 free plants. He reports no F<sub>3</sub> progeny tests.

Recently Mains and Dietz<sup>3</sup> isolated five physiologic forms of *E. graminis hordei*. These were distinguished by the response of four varieties of barley as follows:

Nepal C. I. 595 resistant (type 1-2)	p.f. 1
Nepal C. I. 595 susceptible (type 4)	
Peruvian C. I. 935 susceptible (type 3-4)	
Goldfoil C. I. 935 susceptible (type 4)	p.f. 5
Goldfoil C. I. 928 resistant (type 0)	p.f. 3
Peruvian C. I. 935 resistant (type 0-1)	
Blackhull-less C. I. 666 resistant (type 1-2)	p.f. 2
Blackhull-less C. I. 666 susceptible (type 4)	p.f. 4

Although the discovery of physiologic specialization complicates the production of a resistant variety of barley by hybridization, such an attempt is being made because of the economic importance of barley mildew. This paper deals with the varietal response and inheritance of resistance to a single physiologic form.

<sup>1</sup>The writer wishes to gratefully acknowledge the assistance of Dr. I. E. Melhus in the preparation of this manuscript.

<sup>2</sup>Biffen, B. H., 1907, Jour. Agr. Sci., 2:109-129.

<sup>3</sup>Mains, E. B., and S. M. Dietz, 1930, Phytopathology 20:229-239.

## MATERIALS AND METHODS

Of the 90 varieties of barley whose reactions to mildew are reported in this paper, 88 varieties were secured from E. B. Mains, Purdue Agricultural Experiment Station, and two from Charles Marsh of Minnesota. The five varieties of barley used as parents of hybrids, together with their response to *Erysiphe graminis hordei* p.f. 4, are as follows: Chevalier C. I. 156 (susceptible, type 4), Goldfoil C. I. 928 (resistant, type 0), Odessa C. I. 927 (susceptible, type 4), Triebi C. I. 936 (susceptible, type 4) and Velvet C. I. 4252 (susceptible, type 4).

Physiologic form 4 was isolated from varieties of barley that served as differential hosts. This form was collected in the field at Moscow, Idaho, by the writer in 1926, by J. Milford Raeder in 1927, and at Davis, California, by Lysle D. Leach in 1927, 1928 and 1929. At the present time it is known to occur only in western United States.

The following hybrids and their reciprocals were made in the greenhouse at Ames, Iowa, during the winter of 1926: Chevalier C. I. 156 x Goldfoil C. I. 928; Triebi C. I. 936 x Goldfoil C. I. 928; Odessa C. I. 927 x Goldfoil C. I. 928, and Goldfoil C. I. 928 x Velvet C. I. 4252. The 22 F<sub>1</sub> plants were not exposed to infection with powdery mildew and were matured in the field during 1927. During the spring of 1928, each individual F<sub>1</sub> seed was planted in a three-inch pot in the greenhouse. The resulting plants were transplanted to the field after being exposed to infection with powdery mildew and their reaction noted. During the spring of 1929, the F<sub>3</sub> progeny tests were made in the greenhouse by planting the seed from each F<sub>2</sub> plant in separate rows two inches apart.

When the seedling hybrids had produced their second and third leaves, they were exposed to infection by atomizing with water and shaking heavily mildewed plants over them. These seedling plants were incubated over night—a period of twelve hours—in a moist chamber at 60-65° F. with a relative humidity of 85-90. The plants exposed to infection remained in the opened chamber until 4 p. m. of the same day, when they were removed to the greenhouse bench.

The reaction of the hybrids was recorded when the powdery mildew had reached its maximum development, this being usually in 8 to 12 days. In order to avoid classifying escaped plants as resistant, all plants recorded as resistant were again exposed to infection.

The types of infection were divided into five arbitrary classes of host reaction. Three of the classes, designated by the symbols 0, 1 and 2, were considered resistant, and two, symbols 3 and 4, were considered susceptible. The five classes and types of infection are shown in table 1.

The parents used in crossing were sharply contrasted, the resistant plant was read as type 0, and the susceptible one, type 4. The hybrids, too, were sharply contrasted, those classed as resistant were read types 0 to 1, and susceptible, types 3+ to 4.

THE RESPONSE OF VARIETIES OF BARLEY TO *ERYSIPHE GRAMINIS HORDEI* P. F. 4

Three plantings of the 90 pure line varieties of barley were each exposed to infection with *Erysiphe graminis hordei* p.f. 4. Classifying resistance as types 0 to 2 and susceptibility as types 3 to 4, 62 varieties were susceptible and 28 resistant (table 2).



TABLE 1. *Classes of reaction of barley to powdery mildew, Erysiphe graminis hordei*

Symbol	Classes of host reaction	Types of infection
0	Highly resistant	Macroscopically, no mildew. Chlorotic or necrotic flecks formed in some varieties. Microscopically sometimes a slight development of mycelia and haustoria.
1	Very resistant	Slight development of mycelia with little or no sporulation. Chlorotic or necrotic areas in some varieties.
2	Moderately resistant	Moderate to abundant development of mycelia with slight sporulation. Chlorotic or necrotic areas in some varieties.
3	Moderately susceptible	Moderate to abundant development of mycelia with moderate sporulation.
4	Very susceptible	Abundant development of mycelia and abundant sporulation.

Goldfoil C. I. 928, Unnamed C. I. 96, Hanna C. I. 906 and Duplex C. I. 2433 are particularly interesting as they gave an 0 type of reaction. The following good commercial varieties are completely susceptible: Horsford C. I. 610, Chevalier C. I. 278, Hanchen C. I. 531, Manchuria C. I. 245, Nepal C. I. 475, Odessa C. I. 927, Oderbrucker C. I. 940, Triebi C. I. 936 and Velvet C. I. 4252.

#### INHERITANCE OF RESISTANCE

*Erysiphe graminis hordei* is a limiting factor in many sections of the United States. Apparently there is considerably less damage in Iowa, however, than in adjoining states. It would be highly desirable to produce varieties of barley resistant to all physiologic forms, but first it is necessary to make a more comprehensive survey for physiologic forms in addition to those known, and further, to ascertain their geographic distribution.

As stated earlier, the  $F_1$  hybrids were not exposed to infection, since a severe reduction in yield might result if susceptibility were dominant.

#### MILDEW REACTION OF THE $F_2$ PLANTS

Seven hundred ninety  $F_2$  plants were secured from the 22  $F_1$  plants, the latter being the product of four different parental combinations. Of the total number of  $F_2$  plants, 588 were susceptible to *E. graminis hordei* p.f. 4 and 202 resistant (table 3). Interpreting this on the basis of a 3:1 ratio, the calculated number of susceptible plants would be 592.5 and resistant 197.5. This leaves a deviation of 4.5 plants, between the observed and calculated results. The  $F_2$  ratio of each cross approximates a 3:1 ratio, although the numbers in some cases are small.

The largest number of  $F_2$  plants from a single cross was derived from Odessa x Goldfoil. A total of 554  $F_2$  plants was secured; 421 were susceptible and 133 were resistant. On the basis of a 3:1 ratio, a deviation of 5.5 plants between the observed and calculated numbers occurred.

The reaction of all  $F_2$  plants to physiologic form 4 was so definite that only one  $F_2$  plant was misclassified on the basis of the  $F_3$  progeny test.

The absence of intermediate types of reaction allowed an easy classification of the hybrid response.

The foregoing results strongly indicate that susceptibility to *Erysiphe graminis hordei* p.f. 4 is dominant, and is caused by a single pair of factors. This indication is further verified by the F<sub>3</sub> progeny tests.

TABLE 2. *Reaction of barley varieties to Erysiphe graminis hordei p.f. 4*

Variety	No. C. I.	Reaction	Variety	No. C. I.	Reaction
Abyssinia	362	2	Hooded Spring	716	4
Abyssinia	1234	2	Horn	866	4
Arequipa	1256	2	Huwan	1080	3
Arlington Awnless	702	1	Juliaca	1114	4
Barquis	1076	3	Kinberly	1382	2
Blackhull	596	4	Kwan	1016	3
"	878	1	Leh	700	3
Blackhull-less	666	3	Lihor	866	4
"	1032	4	Luth	922	1
"	1097	4	Lynch	119	1
Bohemian	204	4	Manchuria	245	4
Bolivia		4	Nepal	475	4
"	1257	2-3	"	489	4
Cabeza	1437	2	"	595	4
Chevalier	156	4	"	598	4
"	278	4	"	724	3
Chile Common	663	1-2	"	1290	4
Chilean C.	1432	2	"	1292	4
Chilean D.	1433	2	Nigrata	2444	1
Club Mariout	261	3-4	Oderbrucker	940	4
Coast	690	2	"	969	4
Consul	1061	3	Odessa	182	3
Country Barley	276	2	"	927	4
Czech	1023	3	"	961	4
Duplex	2433	0	Oswong	697	2
Eagle	913	4	Palestine	939	1
Evans	621	3	Pannier	1330	4
Featherston	911	4	Peru	653	3
"	1120	4	Peruvian	935	1
Gatami	575	4	"	1131	1
"	1413	4	Poda	652	3
Gehangir	1089	3	Pontius	731	3
Goldfoil	928	0	Princess	603	4
Hanchen	531	4	Purple Nepal	1373	4
Hanna	30	3	Quinn	1024	3
"	203	4	Striegum	47	4
"	906	0	Svanhals	187	4
"	942	4	Trebi	936	4
Hansees Hull-less	703	1	Tonot	1012	2
Horsford	507	4	Turkestan	711	1
"	610	4	Velvet	4252	4
Heil Hanna 1.	681	4	Wider	1012	2
Heil Hanna 3.	682	3-4	Zun Pohn Mugi		3
Himalaya	254	4	Unnamed	96	0
"	620	4	Unnamed	2416	2
Hiscin	1053	3			

TABLE 3. *Reaction to physiologic form 4 of F<sub>2</sub> plants from crosses between resistant and susceptible varieties*

Crosses	Hybrid no.	Number F <sub>2</sub> plants	
		Susceptible	Resistant
Chevalier C. I. 156 x Goldfoil C. I. 928	2-1	65	23
Trebi C. I. 936 x Goldfoil C. I. 928	4-1	8	4
" " " "	5-1	17	10
" " " "	5-2	14	5
" " " "	5-3	5	2
" " " "	5-4	3	2
" " " "	5-6	12	2
" " " "	5-7	19	3
Odessa C. I. 927 x Goldfoil C. I. 928	8-1	33	13
" " " "	8-2	69	20
" " " "	8-3	23	10
" " " "	8-4	29	8
" " " "	8-5	46	14
" " " "	8-6	39	8
" " " "	8-7	25	10
" " " "	8-8	15	9
" " " "	8-9	38	8
" " " "	8-10	37	9
" " " "	8-12	25	7
" " " "	8-13	9	3
" " " "	8-14	33	14
Goldfoil C. I. 928 x Velvet C. I. 4252	9-1	24	18
Observed totals		588	202
Calculated totals		592.5	197.5
Deviations		4.5	4.5

MILDEW REACTION OF THE F<sub>2</sub> PLANTS

Although the observed F<sub>2</sub> was close to the expectation and was entirely free from plants giving an intermediate reaction to powdery mildew (Plate 1), the F<sub>3</sub> progeny tests confirmed the reaction of the F<sub>2</sub>.

It was impossible to continue all F<sub>2</sub> plants through the F<sub>3</sub> progeny tests, because the individual F<sub>2</sub> plants were transplanted from the three-inch pots to the field in June and many did not mature. All the matured F<sub>2</sub> plants (126 in number) were harvested and used in these tests. These plants produced a total of 1,263 F<sub>3</sub> plants.

Forty-nine susceptible F<sub>2</sub> plants produced 414 susceptible F<sub>3</sub> and no resistant plants. These results are shown in table 4. Fifty-two susceptible F<sub>2</sub> plants produced 436 susceptible F<sub>3</sub> and 152 resistant F<sub>3</sub> plants. On the basis of a 3:1 ratio, the calculated number is 441:147 or a deviation of five plants from the actual results. Twenty-five resistant F<sub>2</sub> plants were apparently homozygous for resistance as they produced 261 resistant F<sub>3</sub> plants.

The largest number of F<sub>3</sub> individuals was the 1044 F<sub>3</sub> from 94 F<sub>2</sub> plants of the cross Odessa C.I. 927 x Goldfoil C.I. 928 (table 4). Forty-two susceptible F<sub>2</sub> plants were homozygous-susceptible, giving 372 susceptible F<sub>3</sub> plants. Forty-two susceptible F<sub>2</sub> segregated into 365 susceptible, to 122 resistant plants; 20 resistant F<sub>2</sub> plants were homozygous-resistant as shown by their 185 resistant F<sub>3</sub> progeny.

TABLE 4. Breeding behavior for mildew reaction of  $F_3$  progenies of barley grown from seed of individual  $F_2$  plants of crosses between susceptible and resistant varieties

Cross	Hybrid no.	No. homozygous- susceptible		No. heterozygous			No. homozygous- resistant	
		Pro- genies	Plants	Pro- genies	Plants		Pro- genies	Plants
					Sus.	Res.		
Chevalier C.I. 156 x Goldfoil C.I. 928 Trebil C.I. 936 x Goldfoil C.I. 928 "	2-1	1	4	1	9	3	2	51
	4-1	3	12	4	30	10	1	9
	5-1				12	10	1	3
	5-2	1	8	3			1	13
	5-3	1	2					
	5-6	1	16	2	20	7		
	5-7	1						
Odessa C.I. 927 x Goldfoil C.I. 928 "	8-1	4	35	5	29	11	2	10
	8-2	7	135	4	48	15	2	19
	8-3	2	14	6	39	10	1	9
	8-4	4	39	3	17	8	2	25
	8-5	2	9	9	105	37	2	32
	8-6	1	3	3	26	9		
	8-7	1	5					
	8-8	3	20	6	45	19	4	26
	8-9	7	38	3	29	5	4	23
	8-10	8	46				1	11
	8-12	1	10	1	12	4		
	8-13	1	13					
	8-14	1	5	2	15	4	2	30
Totals		49	414	52	436	152	25	261



## SUMMARY

The response of 90 pure line varieties of barley to *Erysiphe graminis hordei* p.f. 4 was determined. The following four varieties were highly resistant: Goldfoil C.I. 928, Unnamed C.I. 96, Hanna C.I. 906 and Duplex C.I. 2433. Forty-four varieties were very susceptible and the other 42 were intermediate in reaction.

Susceptibility seems to be due to a single pair of factors as shown by the close approximation to a 3:1 ratio in the  $F_2$  and verified by the  $F_3$  progeny tests.

## PLATE I

An F<sub>2</sub> plant resistant (left) to *Erysiphe graminis hordei* p. f. 4 and susceptible (right) of a cross Goldfoil C. I. 928 x Velvet C. I. 4252. This definite segregation is typical of the hybrid reaction to this physiologic form of powdery mildew.



PLATE I





# EXPERIMENTS ON THE BIOLOGY OF INFUSORIA INHABITING THE RUMEN OF GOATS<sup>1</sup>

HELEN A. MOWEY WITH ELERY R. BECKER<sup>2</sup>

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*From the Department of Zoology, Iowa State College*

## INTRODUCTION

In 1843, Gruby and Delafond, while performing experiments on digestion, discovered the protozoan fauna of the rumen and reticulum of domesticated ruminants. The main interest in the rumen infusoria centered at first in their morphology and classification, with occasional opinions expressed as to the physiological rôle in the host animals. Later investigations have dealt mainly with the latter phase of the subject. In a recent paper by Becker, Schulz and Emmerson (1930), the various views regarding the value of the rumen infusoria to their hosts have been discussed and the literature cited. They mention the following six outstanding opinions as to the significance of the presence of infusoria in the rumen: (1) The rumen infusoria convert plant substances within their bodies into animal substances which are more readily available to the digestion of the host; (2) they are useful as scavengers in reducing the numbers of bacteria and moulds that multiply in the rumen of the ruminants (bacteriophagic activities); (3) they are injurious parasites, causing pathological conditions in the digestive canal; (4) they aid mechanically in mixing the rumen contents; (5) they digest considerable amounts of cellulose, thus making it available to the host animals; (6) they are present merely as harmless commensals. In the course of their experiments, Becker, Schulz and Emmerson arrived at the conclusion that, until further investigations have definitely proved otherwise, the rumen infusoria must be regarded as mere harmless commensals. The research reported in this paper represents another method of approach to the problem of the significance of the host-parasite relationship in the instance under consideration.

## REVIEW OF LITERATURE

Ferber (1928 and 1929) did some important work in determining the numbers of infusoria normally inhabiting the rumen under normal feeding conditions. For goats in a healthy condition and with normal feeding the number approximated 800 to 1,000 infusoria per cubic millimeter of rumen contents, or practically 1,000,000 per cubic centimeter.

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For counting the infusoria he used a Fuchs-Rosenthal blood-corpuscle counting chamber. He found that the infusoria were extraordinarily sensitive to unfavorable conditions of nourishment. They disappeared within three or four days when the host was starved, but reappeared after four or five days with resumption of feeding. The infusoria seemed to disappear always in a certain order and to reappear in the reverse order of the disappearance. The infusoria appeared in lambs and kids only after they commenced to eat hay. In feeding adult animals with hay and water alone, however, the number of infusoria declined, only to rise again with the addition of more nourishing foodstuffs. Starch grains were apparently digested in the endoplasm through the agency of enzymes secreted by the rumen infusoria themselves, but the fat droplets of milk were digested inside the bodies of the infusoria through the action of the bacteria which had been ingested from the surrounding medium.

It has been repeatedly observed that the infusoria inhabit only the first two pouches of the ruminant stomach, and that those which pass into the third and fourth sections of the stomach are digested. With this fact in mind, Ferber (1928) experimented with the possibility of the infusoria utilizing non-albuminous foodstuffs and converting them into the protein of their own bodies, and thus increasing by this synthetic process the protein supply of their hosts. He fed the ruminants food rich in carbohydrates, but poor in protein, supplemented by the amides ammonium acetate and urea. He found that the infusoria could not use nitrogen-containing substances of a non-protein nature in the synthesis of their own protoplasm, for there was no subsequent increase in numbers such as followed the use of proteins as supplementary foods. Therefore, he denied the hypothesis that the infusoria synthesize proteins from amides.

He did believe, however, that the host derived benefit from the process of converting plant protein, difficult to digest, into animal protein, supposedly more easily digested. Certain physiological conditions of the hosts evidently call for increased protein metabolism. To help meet these requirements, there is an increase in the numbers of infusoria in the rumen.

How the physiological changes in the host condition the reproduction of the infusoria Ferber did not explain, but he assigned to the infusoria a symbiotic relationship with their ruminant hosts. This view was upheld by Mangold (1929,b), an eminent physiologist, who stated that the rumen infusoria played an important rôle in the nourishment of their host animals in that they transformed the plant protein, which is difficult to digest, into animal (infusorian) protein, which is more easily digested. In return for this service, the infusoria receive protection, shelter and food within the body of the host. This reciprocity represents a typical symbiosis.

At Mangold's instigation, Ferber (1929, a) carried on further experiments as to the effects on the infusoria of variations in the protein metabolism of the host animals. He found that, during the last six weeks of pregnancy and during the lactation period, the number of infusoria in goats and sheep increased to double the usual number—that is, to around 2,000 per cmm. of rumen contents, and that, after lactation ceased the infusoria returned to the normal number of about 1,000 per cmm. This heavy increase in the infusoria numbers concomitant with the increased

protein metabolism of the host, he affirms, represents the contribution of the infusoria to the support of this increased protein metabolism.

Ferber included in his investigation observations on young, growing lambs and kids in which there is, of course, a more active protein metabolism than when the mature condition is attained. The infusoria appear in the rumens of the lambs and kids coincident with the consumption of hay as food, before the cessation of suckling. During the period of rapid growth of the kids, especially from the fifth to the seventh or eighth month, the infusoria increase to double the normal number, and then, with the diminishing growth rate of the host, decrease to normal with the attainment of the adult size. The situation is thus analogous to that in pregnant and lactating animals. Experiments were performed also upon lambs of the same age, but in different conditions of nourishment. For these observations Ferber employed lambs on pasture. At the time of obtaining the rumen samples, he noted whether the lambs, in the judgment of the shepherd who cared for and knew them, were in a condition of good, medium or poor nourishment. Here again it was found that high numbers of infusoria coincide with good and medium states of nutrition, and that poorly nourished animals have the lowest population of rumen infusoria. Still further, he found that the nourishment condition and infusoria numbers were still in accord in animals of varying ages, with, in general, a decrease in infusorian numbers accompanying an increase in age. In normally nourished, slow-growing, or adult animals, the medium, normal number of infusoria was fairly constant, around 1,000 per cmm. of rumen contents.

Ferber deduced from these results that there must be an optimal condition of the rumen contents for the well-being of the animal, and that this condition was reflected in the number of infusoria present therein. He, accordingly, investigated the relationship between the rumen infusoria and the density of the rumen content (1929, b). The conditions of density of the rumen contents were designated as "normal", "thick", "thin", "very thick", and "very thin". The results yielded some irregularities, but in general with a "normal" density, the number of infusoria were "normal"; with "thick" contents, there was an increase in infusoria; with "thin", there was a decrease. The hydrogen ion factor also entered here, the pH with normal and increased numbers of infusoria being around 7.9; while a drop to the acid side, even to 6.9, accompanied a great loss in the infusorian population. It was inferred, therefore, that there is an optimal condition of the rumen contents in which the infusoria, as well as the host animals, find the most favorable conditions. He further stated that, since in any instance of symbiosis the optimal success of one member is dependent upon that of the other, the number of infusoria could be considered as an indicator of the well-being of the host. He felt that the consistency of the rumen contents and the protozoan population therein were of interest for the practical nutrition of animals, for with favorable cooperation and regulation of these factors, their highest possible productive capacities could be reached.

Inspired by the experiments of Schwarz (1925), who also held to the theory of a symbiotic relationship between the infusoria and their hosts, Ferber and Winogradowa-Fedorowa (1929) tried to achieve a quantitative



determination of the amount of the rumen infusoria. When Gruby and Delafond (1843) discovered the presence of the infusoria in the rumen of ruminants, they estimated that the weight of the protozoa equalled approximately one-fifth of the total weight of the rumen content. Ferber and Winogradowa-Fedorowa found that the total mass of infusoria amounted to a twelfth to a twentieth part of the entire rumen content, and that the entire infusorian nitrogen amounted to from 10 to 20 per cent of the nitrogen present in the rumen. From these results Ferber computed the infusorian protein mass to be 155.6 g. per 100 kg. of rumen contents, in contrast to the higher figure of 256 g. as given by Schwarz. Since the number of infusoria remained fairly constant, and they found an average of seven per cent division forms each day, they drew the conclusion that seven per cent of the total number of infusoria must disappear daily to be digested in the psalterium. They computed therefrom that the rumen infusoria yielded daily to their host animals (sheep and goats) an average of 0.327 g. of protein and that the infusorian protein digested by the host amounted to about two per cent of the total protein digested daily. After three days of starvation of the host, the infusoria disappeared, and, in reappearing with resumed feeding, divided most rapidly on the first day, with the division rate then gradually declining again to the normal percentage of seven per cent.

In presenting the subject of nutrition and digestion in ruminants, Mangold (1929, a) reiterates the importance of the rumen infusoria for their host animals. He agrees that the infusoria reduce cellulose, and show an active carbohydrate assimilation, digesting both starch (Trier, 1926) and fat (Ferber, 1928); that they facilitate protein utilization by transforming plant protein into the more easily digestible animal protein of their own body substance; that the infusoria need protein and are not to be satisfied with amides which they are not themselves able to manufacture into protein. In addition, Mangold (1929, b) states that the number of infusoria within the rumen of the host is limited in a physiological manner under ordinary conditions of good health in the host animal. He considers that the symbiotic relationship between the rumen infusoria and the ruminants has practically been proved. He feels that, since the parallel between the amount of protein metabolism of the host and the infusoria number is an incontestable fact, the increased protein requirement of the host primarily causes the increase in the number of protozoa. But he also states that the physiological connection between the two is as yet unexplained, and that there remains yet to be solved what physiological changes occur in the rumen which, as soon as the protein metabolism increases, cause the rise in the number of infusoria; and conversely, what physiological factors, in the case of decrease of the protein metabolism, cause the lowering of the infusoria numbers.

## THE INVESTIGATION

### PURPOSE OF THE RESEARCH

The investigation was undertaken in order to make additional contributions to facts already known concerning conditions which regulate the increase or decrease of infusorian population in the paunch of the ruminant stomach. Although practical applications of the results were not



foremost in mind at the time of selecting the problem, these would, of course, be evident in case it should be shown that the infusoria in question are material aids to their hosts. Finally, the investigation was planned with the view that a critical analysis and interpretation of the results might yield information either confirmatory or otherwise to the views of Ferber and of Mangold that the relationship between host and parasite is one of symbiosis or mutual aid. A reinvestigation of the problem from the standpoint of the biology of the infusoria was necessary, especially in view of the results in general contradictory to these claims obtained by Becker, Schulz, and Emmerson (1930) and by Becker and Everett (1930).

#### METHOD OF PROCEDURE

##### *Statement of the Problem*

The general problem is stated in the preceding paragraph. More explicitly, the various aspects of the problem were the determination of the following points: (1) the effects due to kind and amounts of a variety of food materials upon the numbers of infusoria; (2) the effects of the food upon the pH of rumen contents and the consequent effect upon the infusoria; and (3) the relationship between the amount of sediment and the number of infusoria.

For these experiments three goats were used, one female of about four to five years of age, and two males, each of about one to two years. In the tables, the female goat is designated as Goat I, and the two males as Goat II and Goat III, the latter being the larger of the two animals. The female goat freshened during the course of the investigation, giving birth to two kids. Thus we were enabled to obtain data during the periods of pregnancy and lactation of this one goat.

*Species of protozoa present.* The infusoria present in the three goats varied somewhat as follows: In Goat I, *Diplodinium multivesiculatum*, *D. ecaudatum*, *D. hamatum*, *Entodinium simplex*, *E. minimum*, *E. caudatum*, *E. furca*, and *E. bicarinatum*; in Goat II, *D. multivesiculatum*, *D. bursa*, *D. ecaudatum*, *E. caudatum*, *E. bicarinatum*, *E. simplex*, and *E. minimum*; while in Goat III there were still fewer species, namely, *D. multivesiculatum*, *D. ecaudatum*, and *E. minimum*, with a few *E. simplex*, and very rarely an *E. caudatum*. About the middle of October, 1929, the species of *Diplodinium* above mentioned disappeared in all three goats, except *D. multivesiculatum*, which was, from then on, the only species of *Diplodinium* represented. The *Entodinium* fauna remained about the same for each goat throughout the period of the experiments. The genera *Ophryoscolex*, *Isotricha*, *Dasytricha* and *Buetschlia* were not represented in the infusorian fauna of the three goats used in these investigations.

##### *Experimental Methods*

*Obtaining samples.* In obtaining samples of rumen contents for examination, the jaws of the goat were held apart by means of a wooden block inserted across the mouth between the teeth. A rubber tube (horse catheter) was passed through a hole in the center of the block and pushed down the oesophagus into the rumen. Care had to be exercised that the tube did not pass into the trachea. When in the rumen, suction was ap-

plied to the tube, and the sample of rumen contents thus obtained was expelled into a bottle.

*Feeding.* The hay and grain were eaten readily by the goats. It was necessary, however, to prepare and inject into the goats the other materials used, in order to assure the intake of a definite quantity of them. A suspension was made by mixing and stirring the material with tap water. This was passed into the rumen through a funnel connected to a rubber tube. The asparagin was ground in a mortar, but did not go into solution when water was added. The fine particles were washed through the tube into the rumen with water.

*Determining pH.* Immediately upon reaching the laboratory, the sample of rumen contents was thoroughly shaken and a definite quantity transferred to a vial by means of a marked pipette. This sample was then diluted with twice the volume of distilled water. The two liquids were thoroughly mixed, and the hydrogen ion concentration of the diluted rumen content was determined at first by both the La Motte Roulette comparator and the drop method. The results obtained compared so closely that after a few weeks only the drop method, which was quicker and easier, was used. Phenol red was the indicator employed throughout.

*Counting and computing.* For counting the infusoria, a Max Levy haemocytometer and an ocular micrometer calibrated to the microscope used were employed. From the thoroughly mixed mass of rumen content and distilled water a drop was transferred, by means of a pipette, to the counting chamber, and the infusoria which fell within the square marked on the ocular micrometer were counted. Account was taken only of the numbers of the two genera, *Diplodinium* and *Entodinium*, present. The counting chamber was shifted so that counts were made from six different locations in each drop. The chamber was refilled four times, giving a total of twenty-four counts for each sample of rumen content. The total numbers of *Diplodinium* and of *Entodinium* thus found were multiplied by three to account for the dilution of the sample, and then by six and two-thirds, which was necessary, according to the calibration previously determined, to express the total amount in cubic millimeters. Since twenty-four counts were taken, the average number per cubic millimeter was obtained by dividing the total amount by twenty-four, and the average number per cubic centimeter by multiplying by 1,000.

*Determining percentage of sediment.* The remainder of the sample of rumen content which was not diluted and used in the counting was poured into a test-tube and left to stand, so that a sediment would form. The percentage by volume of sediment to the total amount was then calculated.

*Determining volume of protozoa.* Measurements of fifty *Entodinia* were made. The specimens were taken as they were encountered with no regard for species or for size. Fifty *Diplodinium multivesiculatum* were measured. An ocular micrometer which had been calibrated to the microscope was employed. The specimens were measured for length, width and thickness. Length and breadth were easy to measure, but to obtain the thickness it was necessary to tap the cover glass gently until the specimen being measured was turned. After securing the fifty sets of measurements for each genus, the averages were computed for the three dimensions for both genera. Models of plastic clay were then constructed according to

scale for the average measurements of the two genera, and the volumes of each determined by the displacement of water.

## PRESENTATION OF DATA

*Different Feeds and Tables of Results*

*Effects on numbers of protozoa of feeding fresh green plants.* At first the goats were allowed to graze on blue grass within the confines of a pen. While on this feed, they drank little or no water. Table 1-a shows the numbers of infusoria after several days of this diet. The experiments were begun on Goat I in August, 1929, while Goats II and III were not used until September, 1929. In most cases the diet given to one goat at one time was repeated upon another at a later date, if not during the same interval. Table 1-b shows the results of feeding green alfalfa. With these feedings on green fodder, the infusoria number remained surprisingly low, averaging between 250,000 and 300,000 per cc.

*Effects with green fodder and grain.* Goats II and III were given 500 grams of grain mixture in addition to the blue grass on which they grazed at will. The grain mixture used consisted of 100 parts of ground corn, 100 parts of ground oats, 50 parts of wheat bran, 10 parts of linseed

TABLE 1 *Effects of feeding fresh green plants*(a) Blue grass *ad libitum*

Day	Goat	pH	No. of Entodinia per cc.	Av. petg. of vol. per cc.	No. of Diplodinia per cc.	Av. petg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total petg. of vol. per cc.
9th	I	8.0	232,915		50,416		283,331		
10th	I	7.8	177,500	0.381	18,333	6.312	195,833	239,582	6.693
5th	II	7.5	189,167		7,083		196,250		
6th	II	7.6	162,500		10,000		172,500		
8th	II	7.8	265,417		23,333		288,750		
9th	II	7.6	267,917	0.411	35,000	3.462	302,917	240,104	3.873
5th	III	7.6	270,000		4,167		274,167		
6th	III	7.4	296,667		5,000		301,667		
8th	III	7.4	296,250		7,500		303,750		
9th	III	7.1	98,750	0.446	-----	0.765	98,750	244,583	1.211

(b) Green alfalfa *ad libitum*

4th	I	7.8	360,833		55,000		415,833		
5th	I	8.0	411,666		60,000		471,666		
7th	I	8.2	360,833		75,000		435,833		
8th	I	8.0	722,916	0.816	110,000	13.772	832,916	539,062	14.633
7th	II	7.6	130,000		14,166		144,166		
8th	II	7.7	259,166		17,500		276,666		
9th	II	7.6	215,833		25,000		240,833		
10th	II	7.6	204,166		30,000		234,166		
11th	II	7.7	207,500	0.377	23,333	4.040	230,833	225,333	4.417

TABLE 2 *Effects of feeding with green fodder and grain*

(a) Blue grass <i>ad libitum</i> and 500 g. grain									
Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. pctg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.
5th	II	7.5	380,833		65,833		446,666		
6th	II	7.4	284,166		89,166		373,332		
8th	II	7.5	147,916		129,166		277,082		
10th	II	7.3	132,500	0.439	11,666	13.581	144,166	310,289	14.020
5th	III	7.2	1,645,833		30,833		1,676,666		
6th	III	7.2	1,215,000		14,166		1,229,166		
8th	III	7.1	776,666		55,833		832,499		
10th	III	7.1	925,833	2.119	15,833	5.356	941,666	1,169,999	7.475
(b) Green alfalfa <i>ad libitum</i> and 1000 g. grain									
22nd	I	7.6	2,999,167		89,167		3,088,334		
23rd	I	7.8	2,447,500		90,833		2,538,333		
25th	I	7.7	3,014,583		115,833		3,130,406		
26th	I	7.5	3,900,833	5.736	173,333	21.538	4,074,166	3,207,812	27.274

oil meal, and some minerals. Table 2-a shows the effects of this combination upon the numbers of protozoa, and table 2-b those of the green alfalfa-grain combination. As these two tables clearly indicate, the addition of the grain mixture to the green feed greatly increased the infusorian population.

*Effects with grain.* Table 3 showing the effects of feeding grain alone, depicts a lowering of the pH, and, after an initial increase, a considerable decrease of the infusorian numbers. The decrease may have been due to the pH, but it should be stated that the goat tired of the diet of grain alone, and, after the first week, ate only about one-half of the 1,000 grams offered her daily.

TABLE 3 *Effects of feeding with grain alone*

1,000 g. grain									
Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. pctg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.
5th	I	6.9	6,707,500		75,000		6,782,500		
6th	I	7.0	7,520,833		76,666		7,597,499		
8th	I	7.0	150,833		9,166		159,999		
10th	I	7.0	497,750	6.903	12,166	7.941	509,916	3,762,478	14.844



The goats were fed for the remainder of the experiments on dried alfalfa hay, used alone, or in conjunction with other foodstuffs. Tap water, unless otherwise stated, was given for drink. The daily count showed that an interval of seven to nine days after the instalment of a new feed was necessary for the infusorian fauna to reach a more or less constant level.

*Effects with alfalfa hay.* As indicated in table 4, the infusorian fauna remained at a lower level with a feed of dried hay than with green fodder, but rose with the addition of grain, increasing with increased amounts of grain.

TABLE 4 *Effects of feeding with alfalfa hay*

(a) 250 g. hay									
Day	Goat	pH	No. of Entodinia per cc.	Av. petg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. petg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total petg. of vol. per cc.
5th	II	7.5	94,166		2,500		96,666		
6th	II	7.9	79,166		4,166		83,332		
7th	II	7.8	115,816		10,816		126,632		
8th	II	7.7	101,666	0.181	4,166	0.534	105,832	103,115	0.175
(b) 500 g. hay									
6th	I	7.6	75,000		15,833		90,833		
7th	I	7.6	112,500		12,500		125,000		
8th	I	7.6	91,666		16,666		108,332		
9th	I	7.6	75,000	0.164	10,000	2.525	85,000	102,791	2.689
7th	II	7.8	206,666		13,333		219,999		
8th	II	7.7	275,833		10,000		285,833		
10th	II	7.7	247,500		10,833		258,333		
11th	II	7.8	270,000		8,333		278,333		
12th	II	7.7	280,833	0.475	5,833	1.775	286,666	265,833	2.250
6th	III	7.2	102,500		1,666		104,166		
7th	III	7.5	99,166		5,000		104,166		
8th	III	7.5	105,000		5,816		110,816		
9th	III	7.5	102,500	1.190	10,833	1.070	113,333	108,120	1.260
(c) 1,000 g. hay									
8th	I	7.8	142,500		21,666		164,166		
10th	I	7.7	95,833		11,666		107,499		
11th	I	7.8	84,166		3,333		87,499		
12th	I	7.7	109,166	0.200	4,166	1.874	113,332	118,124	2.074
8th	III	7.6	137,500		8,333		145,833		
10th	III	7.7	140,833		10,000		150,833		
11th	III	7.6	135,000		5,000		140,000		
12th	III	7.7	192,500	0.281	24,166	2.180	216,666	163,333	2.461

TABLE 5 *Effects of feeding with alfalfa hay and grain*

(a) 400 g. hay and 250 g. grain									
Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. pctg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.
7th	I	7.8	1,903,333		27,500		1,930,833		
8th	I	7.8	1,566,666		40,833		1,607,499		
9th	I	7.8	1,975,833		63,333		2,039,166		
10th	I	7.7	2,043,333	3.474	20,833	7.000	8,064,166	1,910,416	10.474
(b) 500 g. hay and 125 g. grain									
8th	II	7.7	983,333		20,000		1,003,333		
9th	II	7.7	1,265,833		39,166		1,304,999		
10th	II	7.7	1,368,333		25,000		1,393,333		
11th	II	7.7	1,612,500		29,375		1,641,875		
13th	II	7.7	1,622,500	2.524	35,000	5.455	1,657,500	1,400,208	7.979
(c) 500 g. hay and 250 g. grain									
4th	II	7.5	7,795,000		67,500		1,862,500		
5th	II	7.6	1,705,833		62,500		1,768,333		
6th	II	7.6	1,605,000		25,833		1,630,833		
7th	II	7.6	2,884,166	3.475	85,833	11.094	2,469,999	1,932,916	14.569
(d) 1,000 g. hay and 125 g. grain									
8th	I	7.7	687,500		2,500		690,000		
9th	I	7.7	967,500		1,666		969,166		
10th	I	7.7	991,666		3,333		994,999		
11th	I	7.7	890,000				890,000		
13th	I	7.7	597,500	1.534	1,666	0.336	599,166	828,666	1.870
8th	III	7.5	430,833		21,666		452,499		
9th	III	7.5	382,500		17,500		400,000		
10th	III	7.5	382,500		10,000		392,500		
11th	III	7.5	451,666		43,333		494,999		
13th	III	7.5	315,833	0.729	10,416	3.780	326,249	413,249	4.509
(e) 1,000 g. hay and 250 g. grain									
5th	I	7.7	1,423,333		1,666		1,424,999		
6th	I	7.7	1,121,666		2,500		1,124,166		
8th	I	7.7	2,296,666		4,166		2,300,832		
9th	I	7.7	2,230,833	3.281	2,500	0.479	2,233,333	1,770,832	2.778
5th	III	7.5	383,333		71,666		454,999		
6th	III	7.5	352,500		90,833		443,333		
8th	III	7.5	285,000		11,666		296,666		
9th	III	7.5	349,166		37,500		386,666		
10th	III	7.5	468,333	0.682	53,333	9.742	521,666	420,666	10.424
(f) 150 g. hay and 375 g. grain									
7th	III	7.0	810,000		59,166		869,166		
8th	III	7.0	970,000		100,000		1,070,000		
10th	III	7.0	1,540,000		39,166		1,579,116		
11th	III	7.0	2,745,000	2.814	52,500	11.492	2,797,500	1,578,958	14.306

TABLE 5 (Cont.)

(g) 250 g. hay and 375 g. grain									
Day	Goat	pH	No. of Entodinia per cc.	Av. petg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. petg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc. per goat	Total petg. of vol. per cc.
7th	I	7.2	3,124,500		90,833		3,218,333		
8th	I	7.2	3,010,000		105,833		3,115,833		
10th	I	7.2	2,487,500		65,833		2,553,333		
11th	I	7.2	2,881,666	5.338	118,333	17.483	2,999,999	2,971,874	22.821
7th	II	7.0	4,408,333		26,666		4,434,999		
8th	II	7.0	4,465,000		66,666		4,531,666		
10th	II	7.0	5,597,500		86,666		5,684,166		
11th	II	7.0	4,947,500	9.010	52,500	10.673	5,000,000	4,912,707	19.683
(h) 500 g. hay and 375 g. grain									
6th	II	7.6	4,757,500		127,500		4,885,000		
7th	II	7.6	3,314,166		184,166		3,498,322		
8th	II	7.6	2,389,166		123,333		2,512,499		
10th	II	7.6	1,655,000	5.622	70,000	23.183	1,725,000	3,155,205	28.805
6th	III	7.7	2,200,833		40,833		2,241,666		
7th	III	7.7	2,149,166		82,500		2,231,666		
8th	III	7.7	1,367,500		65,000		1,432,500		
10th	III	7.7	1,103,333	3.165	46,666	10.788	1,149,999	1,763,955	13.953
(i) 750 g. hay and 375 g. grain									
6th	I	7.7	1,970,333		14,166		1,984,499		
7th	I	7.8	2,150,000		29,166		2,179,166		
8th	I	7.8	2,288,333		39,166		2,327,499		
10th	I	7.8	1,601,666	3.717	44,166	5.815	1,645,832	2,034,249	9.532
(j) 750 g. hay and 60 g. grain									
8th	I	7.7	815,833		35,833		851,666		
9th	I	7.8	725,833		24,166		749,999		
10th	I	7.8	740,000		34,166		774,166		
11th	I	7.8	746,666	1.405	23,333	4.394	769,999	786,457	5.799
(k) 500 g. hay and 60 g. grain									
8th	II	7.7	1,680,833		60,000		1,740,833		
9th	II	7.8	1,818,333		62,500		1,880,833		
10th	II	7.8	1,540,000		70,833		1,610,833		
11th	II	7.8	1,155,000	2.874	40,000	10.712	1,195,000	1,606,875	13.586
8th	III	7.7	956,666		33,333		989,999		
9th	III	7.8	1,105,000		33,333		1,138,333		
10th	III	7.8	1,081,666		35,000		1,116,666		
11th	III	7.8	903,333	1.877	46,666	6.763	949,999	1,048,249	8.640

*Effects with alfalfa hay and grain.* The different combinations of hay and grain fed are indicated in the different sections of table 5. The drop in numbers shown for both goats in section d may have been occasioned by the small amount of grain in proportion to the hay given as feed. With an increased amount of grain and the same amount of hay,

the numbers rose again for Goat I, which ate all the food given her, but remained low for Goat III, whose appetite was not sufficient at the time to consume the full allotment of his daily feed (section e).

Judging from the results given in sections f, g, h, and i of table 5, 375 grams of grain supplied optimal conditions for the growth of the infusoria, as the numbers remained very high, even with varying amounts of hay. With an increased amount of hay (section h), the numbers remained high. Goat III was especially irregular in his feeding, eating all the hay on some days and very little on others. All of the goats were always

TABLE 6 *Effects of feeding with hay, grain, tap and distilled water*

(a) Hay *ad libitum*, 250 g. grain, and tap water

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Dipodinia per cc.	Av. pctg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.
6th	I	7.7	954,166		85,833		1,039,999		
7th	I	7.9	825,833		51,666		877,499		
8th	I	7.7	1,085,833	1.733	38,333	10.763	1,124,166	1,013,888	12.536
6th	II	7.8	775,000		47,500		822,500		
7th	II	7.8	595,000		50,833		645,833		
8th	II	7.7	1,046,666	1.495	63,333	9.895	1,109,999	859,444	11.390
6th	III	7.0	710,833		4,166		714,999		
7th	III	7.2	598,833		5,833		604,666		
8th	III	7.7	884,166	1.356	23,333	2.040	907,499	742,388	3.396

(b) Hay *ad libitum*, 250 g grain, and distilled water from glass jar

4th	I	7.8	1,057,500		65,833		1,123,333		
6th	I	7.7	797,500		52,500		850,000		
8th	I	7.7	984,166		35,000		1,019,166		
10th	I	7.7	1,270,833	1.907	37,500	8.760	1,308,333	1,075,208	10.667
4th	II	7.7	1,479,166		59,166		1,538,332		
6th	II	7.6	2,448,333		87,500		2,535,833		
8th	II	7.7	2,124,166		102,500		2,226,666		
10th	II	7.6	2,154,166	3.817	97,500	15.868	2,251,666	2,138,124	19.685
4th	III	7.7	839,166		70,833		909,999		
6th	III	7.4	783,333		47,500		830,833		
8th	III	7.6	782,500		49,166		831,666		
10th	III	7.7	600,833	1.394	35,833	9.334	636,666	802,291	10.728

(c) Hay *ad libitum*, 250 g. grain, and distilled water from tin bucket

4th	I	7.6	1,275,833		72,500		1,348,333		
6th	I	7.6	931,666	1.863	43,333	10.635	974,999	1,161,666	12.498
4th	II	7.6	2,595,833		90,833		2,686,666		
6th	II	7.6	2,526,666	4.754	85,833	16.220	2,612,499	2,649,582	20.974
4th	III	7.5	1,056,666		34,166		1,090,832		
6th	III	7.5	848,333	2.716	42,500	7.039	890,833	990,832	9.755



TABLE 7. *Effects of two days of starvation*

No food. Tap water only.

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. pctg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.	Pctg. of sedi-ment
3rd	II	7.3	640,000	1.187	15,833	2.907	655,833	655,833	4.094	40.97
3rd	III	7.6	62,500	0.116	-----	-----	62,500	62,500	0.116	11.58

TABLE 8. *Effects of reinstated feeding with hay and grain*

(a) 227 g. hay and 150 g. grain

Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. pctg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.	Pctg. of sedi-ment
4th	II	7.7	494,166		29,166		523,332			67.35
5th	II	7.7	972,500		59,166		1,031,666			63.64
7th	II	7.7	1,200,000		88,333		1,288,333			50.00
7th	II	7.7	1,408,333		94,166		1,502,499			53.57
8th	II	7.7	1,645,833		76,666		1,722,499			46.15
11th	II	7.6	320,000		79,166		399,166			71.43
12th	II	7.6	372,500		66,666		439,166			94.29
13th	II	7.6	558,333		39,166		597,499			90.91
14th	II	7.5	805,000		68,750		873,750			72.09
15th	II	7.7	1,078,333		51,666		1,129,999			55.56
18th	II	7.7	1,455,833	1.739	61,666	11.929	1,517,499	1,002,309	13.668	45.83
4th	III	7.2	206,666		2,500		209,166			80.65
5th	III	7.4	331,666		17,500		349,166			91.18
6th	III	7.7	299,166		23,333		322,499			-----
7th	III	7.8	380,833		26,666		407,499			51.11
8th	III	7.4	215,833		17,500		233,333			58.33
11th	III	7.7	678,333		39,166		717,499			58.06
12th	III	7.7	830,833		60,000		890,833			51.52
13th	III	7.6	830,833		58,333		663,333			55.19
14th	III	7.5	860,833		50,000		910,833			76.19
15th	III	7.8	438,333		33,333		471,666			43.14
18th	III	7.6	210,833	0.853	14,166	5.717	224,999	490,984	6.570	35.26

(b) 454 g. hay and 300 g. grain

5th	II	7.7	2,805,000		80,000		2,165,000			66.67
8th	II	7.7	2,061,666		60,000		2,121,666			68.00
10th	II	7.7	2,525,000	4.127	70,000	12.854	2,595,000	2,293,888	16.981	48.78
5th	III	7.6	1,500,000		55,000		1,555,000			27.70
8th	III	7.6	1,438,333		61,666		1,499,999			44.64
10th	III	7.6	1,215,000	2.569	41,666	9.691	1,256,666	1,437,222	12.260	37.50

very anxious to receive their grain allotment. With a reduction in the amount of grain given, the protozoan fauna decreased again in number (table 5, sections j and k).

*Effects with alfalfa hay, grain and distilled water.* While performing some previous experiments on rumen infusoria in goats and sheep, Dr. Becker had noticed a decided drop in numbers with the use of distilled water for drink. He mentioned this fact to the writer, and experiments were performed to determine what effect the distilled water would have. Table 6-a shows the numbers of infusorian fauna in the goats before the use of the distilled water for drinking purposes. Section b indicates that the distilled water had no unfavorable effect upon the numbers of protozoa. In his earlier noting of the drop of infusoria numbers with the drinking of distilled water, a tin bucket had been used as the container. In the experiments tabulated in table 6-b the distilled water was administered from a glass jar. As it was thought that the tin bucket might have had some toxic effect previously, one was again used in place of the glass jar. In this case, however, there was apparently no toxic effect from the tin, as the numbers increased, as is indicated in table 6-c. Consequently the deleterious effects attributed to the distilled water must have been due to some other factor.

*Effects of starvation.* Goats II and III were starved for two days after the experiments with the distilled water, and were given nothing but tap water to drink. Table 7 shows the great decrease in the infusoria numbers after the starvation period, and indicates the rapidity with which the protozoan fauna falls off under adverse conditions.

*Effects of reinstated feeding with hay and grain.* With reinstated feeding of hay and grain, the numbers rose again (table 8-a), with some unexplainable drops, however. With a doubling of the food (table 8-b), the numbers increased to approximately the same level as in table 5-h, when slightly more food was given. In both cases, the goats had good appetites, and ate all the food given them.

*Effects with hay.* According to Ferber (1928), hay and water alone as food caused a decrease in the numbers of protozoa. Table 9 shows the agreement of our results with those obtained by him.

*Effects with hay supplemented by cracked corn.* The hay diet was supplemented by carbohydrate food in the form of cornstarch, and this combination was used either alone, or as a basis for additional foodstuffs, until the end of the experiments. There was one exception to this, however, in April, 1930, when Goat III was put on a diet of hay alone, and then of hay supplemented by cracked corn. The infusorian numbers in this case (table 10) practically coincide with those for the apparently optimal amounts of hay and grain. (Compare tables 5-h and 8-b).

*Effects with hay supplemented by carbohydrate food.* The cornstarch used to supplement the hay for the carbohydrate food was Argo Corn Starch, produced by the Corn Products Refining Company of Edgewater, New Jersey. The Research Department of the company very kindly submitted the following average analysis of the corn starch, figured on dry substance: starch, 98.29 per cent; protein, 0.33 per cent; ash, 0.13 per cent; fat, 0.55 per cent; soluble, not protein, 0.07 per cent. From 0.2 per cent to 0.3 per cent pentosans may also be present.

In giving the cornstarch as food, one-half pound of the starch was mixed with about a quart of tap water, and the suspension thus formed

TABLE 9. *Effects of omitting the grain and feeding with hay alone*

681 g. hay										
Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. pctg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.	Pctg. of sedi-ment
13th	II	7.2	280,833		10,000		290,833			32.14
14th	II	7.7	329,166		13,333		342,499			23.53
15th	II	7.7	315,000		17,500		332,500			36.74
17th	II	7.8	249,166	0.545	7,500	2.219	256,666	307,374	2.764	9.21
13th	III	7.2	356,666		28,333		384,999			82.42
14th	III	7.4	250,000		18,333		268,333			38.48
15th	III	7.6	185,000		10,833		195,833			48.57
17th	III	7.7	218,333	0.468	9,166	3.060	227,499	269,166	3.528	23.91

TABLE 10. *Effects of feeding with cracked corn supplementing the hay*

681 g. hay and 340 g. cracked corn										
Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. pctg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.	Pctg. of sedi-ment
8th	III	7.1	1,600,833		2,500		1,603,333			72.00
10th	III	6.7	1,133,333		6,666		1,139,999			45.21
11th	III	6.8	1,616,666		27,500		1,644,166			65.71
13th	III	7.2	862,500		110,000		972,500			78.05
15th	III	7.4	986,666	2.301	147,500	10.803	1,134,166	1,298,833	13.104	-----

TABLE 11. *Effects of feeding with hay supplemented by carbohydrate food*

681 g. hay and 227 g. cornstarch										
Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. pctg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc. per goat	Total pctg. of vol. per cc.	Pctg. of sedi-ment
9th	II	7.7	388,333		38,333		426,666			34.78
10th	II	7.7	315,000		62,500		377,500			15.80
11th	II	7.7	245,000		36,666		281,666			21.62
16th	II	7.8	405,833		86,666		492,499			39.47
17th	II	7.7	652,500		101,666		754,166			62.50
18th	II	7.7	843,333		100,000		943,333			34.88
4th	II	7.8	680,833		22,500		703,833			18.60
5th	II	7.8	905,000		45,000		950,000			23.53
6th	II	7.6	1,135,833		21,666		1,157,499			19.61
8th	II	7.9	1,469,166	1.306	52,500	10.423	1,521,666	760,832	11.729	33.33
9th	III	7.7	536,666		-----		536,666			56.00
10th	III	7.8	585,000		1,666		586,666			69.23
11th	III	7.7	776,666		4,166		780,832			73.53
16th	III	7.6	849,166		23,333		872,499			64.15
17th	III	7.4	584,166		8,333		592,499			40.54
18th	III	7.1	665,000	1.226	34,166	2.193	699,166	678,055	3.419	49.02

poured through a funnel in the end of the stomach tube, and thus into the rumen of the goat. With the addition of the starch to the hay diet the numbers of protozoa increased markedly. (Compare table 2 with tables 9 and 4). Mangold (1929, b) declared that there was no increase in the protozoan fauna with the use of carbohydrate food; that food rich in carbohydrates could in no way compensate for a lack of plant protein which he stated stimulated the infusorian increase. The "starch level" attained in these experiments (see table 11) shows the numbers more than doubled those for the "hay level", although they fall far below those of the level for hay and grain.

*Effects with hay and starch supplemented by protein foodstuffs.*

Following the above experiment, protein foodstuffs were added to the same diet of hay and cornstarch to determine the effects of the proteins upon the numbers of infusoria. The various foodstuffs were mixed with the cornstarch and poured into the animal in suspension as above described. A small quantity of Loeffler's dehydrated beef blood serum increased the protozoan fauna rapidly, in opposition to Mangold's conclusion that it is plant protein which stimulates the increase. With the addition of egg albumin, Merek, impalpable powder, the protozoan number remained at about the "starch level", for Goat II. (Tables 11 and 12-b).

Goat II alone was used for the remaining experiments because Goat III lost his appetite and was discarded. The wheat gluten flour supplementing the hay and cornstarch was very favorable for the development of the infusoria, as the numbers increased to nearly double the numbers on hay and starch alone. Table 12-c shows the increase during this diet, which, however, did not reach the level attained by the same goat with the dehydrated blood serum. Supplementing the feed with asparagin (table 12-d) did not promote the multiplication of the infusoria since the figures obtained fall well within the probable error for starch and hay alone. With casein (table 12-e) the numbers rose higher, though they did not reach the high level obtained with the addition of dehydrated beef blood serum to the hay and cornstarch. To summarize, table 12, with the results of a hay and cornstarch diet supplemented by protein foodstuffs, shows a significant increase in the numbers of protozoa, except in the cases of the egg albumin and of asparagin which is, of course, not a protein.

*Effects of pregnancy.* After the experiments with the distilled water (table 6), Goat I was kept on the same feed for a few days longer, with tap water to drink (table 13-a). Then her food was doubled in amount (table 13-b) to meet the demands of her pregnant condition. This caused an increase in the protozoan numbers, though the number was not consistently double the normal. On February 19, 1930, she gave birth to two kids, one of which survived.

*Effects of lactation.* Table 14 depicts the effects of the lactating period upon the numbers of infusoria in the rumen, and shows that, for this goat, the level during that period was even slightly higher than that indicated by Ferber (1929, a). About the end of April the kid began eating considerable amounts of the mother's grain. This factor had an influence on the protozoan numbers, as table 14-b indicates in the average given under the 112th day. The kid was taken from the mother goat on



TABLE 12. *Effects of feeding with hay and cornstarch supplemented by protein*

(a) 681 g. hay, 227 g. cornstarch and 30 g. Loeffler's dehydrated beef blood serum

Day	Goat	pH	No. of Entodinia per cc.	Av. petg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. petg. of vol. per cc.	Total no. infusoria per cc.	Av. per cc. per goat	Total petg. of vol. per cc.	Petg. of sediment
7th	II	-----	2,836,666		65,833		2,902,499			-----
8th	II	7.5	2,715,000		53,333		2,768,333			-----
10th	II	7.7	2,928,333		35,000		2,963,333			46.15
11th	II	7.8	2,705,000	5.190	45,833	9.181	2,750,833	2,846,249	14.371	33.33
9th	III	7.8	1,614,166		60,000		1,674,166			90.00
10th	III	7.8	1,282,500		50,000		1,332,500			79.16
12th	III	7.9	1,524,166		35,000		1,559,166			48.15
14th	III	7.5	1,210,000	2.612	16,666	7.421	1,226,666	1,448,124	10.033	35.00

(b) 681 g. hay, 227 g. cornstarch and 40 g. albumin egg Merek, impalpable powder

5th	II	7.6	777,500		58,333		835,833			55.00
6th	II	7.8	453,333		29,166		482,499			29.03
8th	II	7.8	355,833		47,500		403,333			1.00
10th	II	7.8	452,500		65,833		518,333			83.87
15th	II	7.8	798,333	1.053	100,000	11.048	898,333	627,666	12.101	52.00

(c) 681 g. hay, 227 g. cornstarch and 45 g. wheat gluten flour

13th	II	7.7	1,301,666		19,166		1,320,832			18.05
14th	II	7.7	1,920,833		87,500		2,008,333			-----
15th	II	7.8	1,691,666		21,666		1,713,332			40.63
16th	II	7.9	1,968,333		37,500		2,005,833			22.22
17th	II	7.9	2,695,833	3.555	69,166	8.630	2,764,999	1,962,666	12.185	57.90

(d) 681 g. hay, 227 g. cornstarch and 28 g. asparagin (an amino acid)

6th	II	7.4	890,000		62,083		952,083			33.33
7th	II	7.4	666,666		61,666		728,332			44.00
8th	II	7.9	838,333		23,333		861,666			25.00
9th	II	7.8	790,833		40,000		830,833			26.31
10th	II	7.6	1,077,500	1.582	54,166	8.860	1,131,666	900,916	10.442	92.11

(e) 681 g. hay, 227 g. cornstarch and 35 g. casein

7th	II	7.7	1,224,166		69,166		1,293,332			-----
8th	II	7.8	1,525,000		25,000		1,550,000			49.00
9th	II	7.7	950,833		13,333		964,166			46.34
10th	II	7.8	1,535,833		24,166		1,559,999			23.33
11th	II	7.7	1,662,500	2.560	10,833	5.233	1,673,333	1,408,166	7.793	40.00

TABLE 13. Effects of pregnancy

(a) 681 g. hay and 250 g. grain

Day	Goat	pH	No. of Ento- dinia per cc.	Av. petg. of vol. per cc.	No. of Diplo- dinia per cc.	Av. petg. of vol. per cc.	Total no. infu- soria per cc.	Av. per cc.	Total petg. of vol. per cc.	Petg. of sediment
6th	I	7.7	1,134,166		61,666		1,195,832			64.29
8th	I	7.8	1,237,500	2.201	40,833	9.411	1,278,333	1,237,082	11.612	51.22

(b) 1,362 g. hay and 500 g. grain

5th	I	7.8	2,064,166		40,000		2,104,166			36.49
6th	I	7.8	1,593,333		25,000		1,618,333			50.79
8th	I	7.8	1,807,500		30,000		1,837,500			43.08
9th	I	7.8	1,894,166		29,166		1,823,332			50.00
12th	I	7.8	1,840,000		56,666		1,896,666			55.17
14th	I	7.8	2,385,833		80,833		2,466,666			70.21
16th	I	7.9	1,450,000		35,000		1,485,000			51.16
19th	I	7.9	1,881,666		80,833		1,962,499			24.00
21st	I	7.9	1,504,166	3.386	94,166	9.624	1,598,332	1,654,721	13.010	52.38

TABLE 14. Effects of lactation

(a) 1,362 g. hay and 500 g. grain

Day	Goat	pH	No. of Ento- dinia per cc.	Av. petg. of vol. per cc.	No. of Diplo- dinia per cc.	Av. petg. of vol. per cc.	Total no. infu- soria per cc.	Av. per cc.	Total petg. of vol. per cc.	Petg. of sediment
3rd	I	7.8	2,361,666		62,500		2,424,166			74.47
13th	I	7.7	2,012,500		40,000		2,052,500			51.85
14th	I	7.7	2,030,833		63,333		2,094,166			62.50
17th	I	7.8	2,775,833		59,166		2,834,999			60.53
22nd	I	7.8	2,773,333		66,666		2,839,999			73.33
27th	I	7.6	2,740,833		48,333		2,789,166			85.45
35th	I	7.7	2,566,666		63,333		2,629,999			40.00
45th	I	7.8	1,822,500		44,166		1,866,666			28.00
50th	I	7.8	2,755,000		71,666		2,826,666			31.25
55th	I	7.8	1,314,166		73,333		1,387,499			44.44
62nd	I	7.8	2,150,833		71,666		2,222,499			51.16
71st	I	7.9	1,898,333	4.207	35,000	10.699	1,933,333	2,325,138	14.906	18.61

(b) Food not entirely consumed. Kid eating some of the grain.

78th	I	7.9	1,049,166		17,500		1,066,666			15.09
83rd	I	7.6	1,590,000		25,833		1,615,833			28.52
90th	I	7.9	1,753,333		38,333		1,791,666			41.03
97th	I	7.9	1,690,000		32,500		1,722,500			20.51
104th	I	7.8	1,277,500		39,166		1,316,666			47.22
112th	I	8.0	2,425,833	3.027	60,833	6.554	2,486,666	1,666,666	9.581	37.04

(c) Kid weaned. Slight milkings.

119th	I	8.0	1,765,000		18,333		1,783,333			35.90
125th	I	8.0	1,648,333	3.167	99,166	10.788	1,747,499	1,765,416	13.955	56.67

the 113th day. Following this separation, however, the mother goat was milked a little each day to relieve the distention of the udder. The milkings were gradually decreased until she was milked no more after the 126th day (table 14-c).

*Effects of cessation of lactation.* As Goat I had not been eating all the food given her, the amount was decreased, as indicated in table 15. It is interesting to note that, previous to the cessation of the lactation period, she had preferred the grain to the hay, but after drying up she left the grain to eat the hay first. Although she was eager to receive food, the amount of hay and grain consumed fell off considerably. With the loss of appetite, and the decrease in the amount of food eaten, the infusorian numbers dropped decidedly, as table 15 indicates.

TABLE 15. *Effects of cessation of lactation*

(a) 200 g. hay and 100 g. grain										
Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. pctg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc.	Total pctg. of vol. per cc.	Pctg. of sedi-ment
7th	I	8.0	550,000		833		550,833			13.83
14th	I	7.7	693,333	1.154	48,333	4.514	741,666	646,249	5.668	64.29

(b) 200 g. hay and 400 g. grain										
Day	Goat	pH	No. of Entodinia per cc.	Av. pctg. of vol. per cc.	No. of Diplo-dinia per cc.	Av. pctg. of vol. per cc.	Total no. infu-soria per cc.	Av. per cc.	Total pctg. of vol. per cc.	Pctg. of sedi-ment
3rd	I	7.6	737,500		7,500		745,000			36.58
11th	I	7.7	1,463,333	2.042	12,500	1.836	1,475,833	1,110,416	3.878	24.14

### Weight of Goats

The goats were weighed to determine whether an increase in protozoan numbers accompanied an increase in weight. The weights were not taken more frequently, as it was clearly apparent that they varied very little. Table 16 shows some weights of the goats.

TABLE 16. *Weights of the goats*

Date	11-18-29	12-20-29	1-24-30
Goat I	60 pounds		
Goat II	47 pounds	50 pounds	47½ pounds
Goat III	60 pounds	60 pounds	55 pounds

Because of her pregnant condition, Goat I was weighed only once. During the period between the first and second weighings, when Goat II gained three pounds, the infusoria numbers rose and fell as is shown in table 5, sections c to k, inclusive, the first weighing taking place at the close of section c, and the second after section k. Goat III, weighed after the feeding indicated in table 5-e, had no increase in weight at the second weighing, but the infusoria numbers rose considerably (table 5-f, h and k). The drop in weight in the two goats on 1-24-30 was due to the fact that

they had been starved for the preceding two days. With the reinstatement of food, they returned to their former weights, which remained about constant, until the illness of Goat III in May, 1930, when he lost weight and was discarded. It was deemed unnecessary to weigh Goats II and III after the last weighing in the table, as there was in both animals no variation in weight apparent enough to be of any importance. Except for the decrease in numbers of protozoa accompanying the loss of weight after the period of starvation, there seemed to be no correlation between the weight of the goats and the numbers of infusoria in these full-grown animals.

### *Effect of pH*

Ferber (1929, b) stated that the hydrogen ion concentration of the rumen content plays a rôle in the development and maintenance of the infusorian fauna. Under normal conditions and infusoria numbers, he observed very slight variations of the pH around 7.9, and, with a lowering of the pH to even so slightly acid as 6.9, he found a heavy depression in the numbers of infusoria. With the three goats used in these experiments, however, 66 per cent of the determinations made fell within the range of 7.6 to 7.8, with 50 per cent of the total at 7.7, with only one-fourth of the remainder of the samples as high as 7.9 to 8.2, and three-fourths 7.5 down to 6.7. This would indicate a slightly lower normal average pH for these three goats than for those used by Ferber, or 7.7 for normal pH in these experiments. For the most part, as is seen by glancing over the tables, the pH and the numbers of infusoria are in general agreement with the correlations as stated by Ferber, but there are a few noticeable discrepancies. For example, in table 2-a, the numbers increased materially in Goat III, while the pH was down to 7.2, and in table 3, with Goat I, the highest levels during the entire series of experiments were reached at a pH of 6.9 and 7.0. However, the sudden drop in numbers immediately following may have been due to the low pH. Again, in table 5-f and g, the numbers were high in all three goats, with a pH as low as 7.2 and 7.0. It might seem, from tables 3 and 5-f and g, that the grain mixture in excess caused the drop in the pH level, as the other sections in table 5 show higher pH levels, with grain, but with more hay in proportion. Table 10 indicates a similar condition with the addition of cracked corn to the diet, for the numbers were high, with a lowered pH. The great drop in numbers and the slightly acid pH for Goat III shown in table 12-b coincide with Ferber's results (1928 and 1929, b). During pregnancy and lactation, the pH remained up. (See tables 13 and 14).

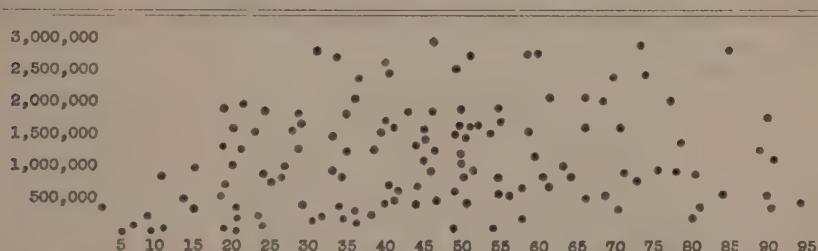
In discussing Ferber's experiments regarding the effect of the pH upon the presence of the infusoria, Mangold (1929, b) stated that the disappearance of the infusoria could not be due to variations in the pH, since, during the starvation experiments with the decrease in infusorian numbers, the pH varied only slightly. Also, with renewed feeding after the starvation periods, the infusoria regained their former numbers by the same pH as that under which they had disappeared. This fact was also apparent in our experiments. (See table 7).



*Effect of Density*

Ferber also stated (1929, b) that the density of the rumen contents played a rôle in governing the infusoria numbers. He designated the various densities of the rumen samples as "normal", "thick", "thin", etc., and found an apparent agreement between the density of the rumen contents and the numbers of infusoria. While the tables (7-on) for these experiments show an occasional agreement between the percentage of sediment and the infusorian numbers, the discrepancies are so numerous that it hardly seems feasible or possible to make any such statement as that of Ferber. For example, in table 8-a, the highest percentages of sediment accompanied the lowest infusorian numbers, and, in section b, with greatly increased counts of protozoa, the samples did not increase in density, but were low in percentage of sediment; also table 12, for the most part, bears out the same lack of agreement. Tables 13 and 14, showing the conditions during pregnancy and lactation, do not indicate the "very thick" densities, according to the numbers of protozoa, that one would expect to find from Ferber's conclusions. An inspection of the correlation chart, table 17, shows an apparent lack of any great degree of correlation between the percentage of sediment and the numbers of infusoria in 132 samples of rumen contents taken from all three goats over a period of five months. It is to be concluded, therefore, that the numbers of protozoa present are not to be correlated with the amount of sediment in the rumen contents.

TABLE 17. *Correlation Chart: percentage of sediment, abscissa; numbers of Infusoria, ordinate*



Winogradowa-Fedorowa and Winogradoff (1929) did not consider the percentage of sediment any indication of the amount of the protozoan fauna. They state that they have found from examinations of the rumen contents of slaughtered animals, that the anterior portion of the rumen contents is more fluid than the posterior. They point out that, since large food particles will not pass through the suction tube while samples are being taken from living animals, only the more fluid portion of the contents is withdrawn. The result is that the sample does not give a true average of the density of the contents. Accordingly, after taking samples of the rumen content of living animals, they added a definite quantity of water to the stomach and took more samples after a stated interval of time. By

a series of mathematical computations they then determined the average density of the rumen content. Their tables showing the differences obtained by the two methods indicate very little variation, however.

Mangold (1929, b), took exception to their results in that they compared the rumen contents of living animals with those of slaughtered ones. In his judgment Ferber's methods were not open to severe censure, as the samples taken were frequently very thick and therefore could not represent merely the more fluid portion of the rumen content. He mentioned further that there is a thorough mixing of the rumen content in the living animal, and that the separation into thicker and thinner portions appears only as a post-mortem variation. He did express the opinion, however, that there is not always a parallel between the number of rumen infusoria and the density of the rumen contents.

### *Rate of Division of Infusoria*

In 1929, Ferber and Winogradowa-Fedorowa published the results of investigations on the division rate of the infusorian fauna in the rumen of sheep and goats. They reasoned that since under normal conditions the protozoan numbers remained fairly constant, the loss from the forms passed on from the rumen with the food material for further digestion must be replaced by the division of some of the remaining forms. In the course of their experiments, they computed an average of seven per cent division forms to the total number counted, and this average remained constant even for two counts per day, taken both before and after the consumption of food by the host animal. Their reasoning, however, is faulty, for the division rate of rumen infusoria is still unknown; and, furthermore, it is quite unlikely that a percentage of a population observed at any moment in the act of dividing represents the amount of reproduction for that day.

In the above experiments, from the first of January, 1930, the numbers of dividing forms were noted and the percentage computed with the total number of the count for the day. Throughout the experiments there were practically no Diplodinia found in a state of division, except in a few rare instances. With a few exceptions, there were some dividing forms of Entodinia found at nearly every counting. But the percentage of recognizable division forms never reached as high as one per cent in any case, and were more often below 0.5 of one per cent than above. Even during the periods of pregnancy and lactation, when a higher percentage of division forms might be expected in accord with the increased fauna, there was little increase in the rate of division, and the total number of forms dividing never amounted even to one per cent of the total number of forms found. It is difficult to estimate the amount of division in this way, however, for only somatic indications of division are recognizable. Stained slides would be necessary to make even fairly accurate estimates of the numbers of dividing forms.

### *Volume of Protozoa*

The average volume of a single specimen for each of the two genera was determined by the displacement of water, as previously described.

From this, the average percentage of volume for each genus was computed for the average with each feed, as is shown in the table. It is of interest to note that the average volume of *Diplodinium multivesiculatum* is approximately one hundred times as great as that of an average Entodinium, the volume of a single average specimen of the latter being 18,560 cubic microns, and that of a single specimen of the former 1,836,320 cubic microns. The average percentage of volume per cc. of rumen contents for each feed is indicated in the tables, and shows that, despite the higher numbers of Entodinia present, the main volume of the infusoria is represented by the Diplodinia. The highest total percentage of volume was obtained in the case of Goat I on a feed of green alfalfa and 1,000 g. of grain, when it reached 27.274 per cent (see table 2-b). The peak reached by the infusoria numbers was for Goat I on the 6th day of feed with 1,000 g. of grain (table 3). The per cent of volume of the infusoria for that day amounted to 28.036 per cent, of which 13.958 per cent represents the volume of Entodinia, and 14.078 per cent that of the Diplodinia. The highest percentage of volume was obtained from Goat I on the 26th day of feed with green alfalfa and 1,000 g. of grain (table 2-b), when the numbers of Diplodinia reached the highest peak throughout the entire course of the experiments. On that day, the volume of the Diplodinia alone reached 31.83 per cent, which, with 7.24 per cent volume of Entodinia, gave a total percentage of volume of 39.07 per cent.

#### DISCUSSION AND SUMMARY

The results obtained in the feeding experiments indicate very clearly that the amount of the infusorian population in the rumen, whether measured by population or by volume, can be manipulated through the food of the animal regardless of its special physiological condition. The numbers of infusoria in adult goats not receiving food fall off rapidly, as has been shown previously by other workers. On a hay diet alone the numbers are comparatively low. The population is augmented by the addition of a small amount of grain to the ration. With each succeeding increase in the grain allotment there is a subsequent rise in numbers of infusoria. Similarly, and contrary to Ferber's experience, it has repeatedly been shown that cornstarch used as a supplementary feed with hay will result in more than a doubling of the numbers of infusoria with a diet of hay alone. The addition of small amounts of suitable materials rich in animal protein, such as dried blood serum, to the hay and starch constituents of the diet will stimulate the reproduction of the infusoria exceedingly.

Parenthetically, it may be noted that it is not known whether these materials regulate the numbers of infusoria directly or indirectly. All attempts by investigators up to the present time to grow holozoic protozoa in purely liquid media have failed. It is possible that an increase in number of infusoria is dependent upon the preliminary development of bacteria at the expense of the protein materials in solution. Nevertheless, whether the development of the protozoan fauna is directly or indirectly conditioned by the food materials introduced into the rumen with the food, the end result is the same. Starch grains are ingested by the infusoria and digested directly by them, as shown by Trier. It has been shown by Dobell that starch stimulates the growth of *Endamoeba histolytica* in cul-



ture. It is not unlikely that other elements in the grain are ingested as solid particles and digested in the endoplasm of the infusoria.

It will be noted that there are many points of agreement in our results with those obtained in Mangold's laboratory. Under conditions of normal feeding there is a fairly constant infusorian fauna of around 1,000,000 organisms per cc. of rumen contents. The numbers fall off rapidly during starvation, and rise with resumed feeding until after about nine days the normal level is attained again. During pregnancy and lactation the numbers are much higher. Upon cessation of lactation, the numbers become much less. Granting, in general, that the data of Ferber and his co-workers are fundamentally correct, how should they be interpreted? Does a concomitant increase or decrease in numbers of infusoria in the stomach of a ruminant at periods of high protein utilization imply causation?

The fallacy of the conclusion that the infusoria are symbionts lies in this very point. It is undoubtedly true that at times of greater protein utilization by the host—such as during growth, pregnancy, or lactation—the numbers of infusoria in the rumen do increase; and conversely, at times of lesser protein utilization—such as during maturity, old age, or after the cessation of lactation—the numbers do decrease. But do these phenomena, as Ferber and Mangold deduce, indicate a condition of symbiosis or mutual aid between the host and parasite? A conclusion to the affirmative, without other more pertinent evidence, could result only from a process of reasoning of the *post hoc, ergo propter hoc* type. It is quite fitting to inquire whether there may not exist a factor other than the physiological condition of the host which will explain the rise and fall of the protein metabolism of the host.

If our results mean anything at all, they indicate that the numbers of infusoria are dependent directly upon the amount and kind of food which the host consumes. The artificial manipulation of the feed of mature, male goats can bring about numbers of infusoria corresponding to those in growing, pregnant, or lactating animals; or on the other hand, similar to those in "drying up", or aged animals.

It is a well known fact, and one so stated in standard works on nutrition, that growing, pregnant, or lactating animals consume more concentrated foods, when these are available, than they would otherwise. This was very evident in the case of Goat I, which became very greedy for concentrates at the onset of and during pregnancy, and also during lactation. At the time of cessation of lactation the grain ration was only partially consumed and much less relished.

Here, then, an explanation of the behavior of the infusorian population at various times in the life history of the ruminant seems to present itself. It is the increase and change in the appetite which results in the animal eating more and richer feeds which leads to an increase in number of rumen infusoria. This supplies an intelligible *modus operandi* for the sequence of events in the infusorian life of the rumen. It is evident to us, after reading most carefully the works of Ferber and of Mangold, that they did not have exactly this factor in mind, for Mangold states explicitly that the mechanism controlling the relationship between physiological need and numbers of infusoria is unknown. The following translation is from



page 176 of his work "Die Verdauung der Wiederkäuer" (1929, b) :—"There must still be solved the question what physiological changes in the rumen of ruminants there are which, as soon as its protein metabolism ascends, cause the increase in the infusoria number, and conversely, what physiological factors in the lowering of the protein metabolism condition the decrease of the infusoria number." And on the same page follows this sentence:—"Experiments seem to have proved that the primary variations always start from the host animal, and the changes of infusoria number represent the secondary appearance."

In face of the admission that the nature of the physiological factors which regulate the numbers of infusoria in the rumen is unknown, any pronouncement to the effect that the relationship between host and parasite is one of symbiosis would seem premature and unwarranted. A critical study of the influence of diet upon the numbers of infusoria shows that the food plays a major rôle in conditioning the numbers of infusoria. A more sound philosophy of the nature of the association would be that it is one of commensalism. The infusoria live up to the margin of subsistence provided for them in the rumen. When the food is scanty or composed largely of plant fiber, the numbers are comparatively small. When there is an abundance of rich and easily digested food present in the rumen, the numbers of infusoria increase tremendously. The infusoria are thus "fair weather friends" of the ruminant, for they abound in numbers when there is an abundance of nutritious food. This philosophy is much more logical than one which implies that the micro-organisms in question unlock reserves of proteins or other food materials difficult to digest by first converting these materials into the substance of their own bodies and then sacrificing themselves to the digestion of the host.

This conclusion that the relationship is one of commensalism agrees with the results obtained by Becker, Schulz and Emmerson (1930) and by Becker and Everett (1930), who compared the digestion and growth in infusoria-free and infected lambs and goats. The comparison was made possible by the development by Becker (1929) of a method of freeing the rumen of infusoria through the administration of a copper sulphate solution.

#### CONCLUSIONS

1. The amount and kind of food consumed are of prime importance in the regulation of the numbers of rumen infusoria.
2. There is no correlation, in adult goats, between the infusoria numbers and the weights of the goats.
3. The percentage of sediment in a sample of rumen contents is no indicator of the amount of the protozoan fauna.
4. The hydrogen ion concentration of the rumen contents evidently has some influence upon the fauna, but it is not a prime factor.
5. The physiological condition of the host regulates the amount of infusoria present in the rumen only in so far as it may affect hunger and appetite.
6. The reactions of the infusoria to conditions in the rumen regulated by the kind and amount of food consumed by the host connote commensalism rather than symbiosis.

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# THE LITERATURE OF ALKYLATED CARBOHYDRATES

## I. TRIOSE, TETROSE AND PENTOSE DERIVATIVES

HAROLD W. COLES

*From the Laboratory of Plant Chemistry, Department of Chemistry, Iowa State College,  
and Lilly Research Laboratories, Indianapolis, Indiana*

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### INTRODUCTION

There is no adequate summary of the rapidly increasing literature of alkylated carbohydrates in American journals. Such summaries are often-times helpful to the busy worker who cannot find time to go through the large amount of literature for the information he is seeking. It is hoped that a series of papers will be of assistance to the worker in this special field.

Alkyl substitutions in the glycosidic position are omitted except for the glycosides of methylated sugars. Decomposition and oxidation products are described along with their parent substances, and the compounds are given their author's description, no attempt being made to conform them to any particular system of nomenclature. The figures in parentheses refer to the bibliography. In listing optical rotations, concentrations are omitted for the sake of space.

### TRIOSES

#### GLYCEROSE

Various methyl ethers of glycerin have been described by Fairbourn (61) in an effort to secure standards of reference in work on the configuration of glycerol derivatives. Glycerol has been methylated to give *monomethyl glycerol* (6, 62), *dimethyl glycerol* (21) and *trimethyl glycerol* (61). *Benzylidene* and *ethylidene methyl glycerol* are described in the literature (57, 58) together with *hexamethyl glycerol glucoside* (21). Dl-glyceraldehyde has been methylated. Various combinations of chlorine and the methyl group in glycerol are described (62).

### TETROSES

#### ERYTHROSE

*Monomethyl d-erythronic acid* (dihydroxy-methoxy-butyric acid) is obtained by oxidation of monomethyl alpha-fructose (4).

2, 4-*Dimethyl d-erythronic acid* (hydroxy-dimethoxy butyric acid) is prepared (37) from an unsaturated compound obtained from the lactol acid of tetramethyl  $\gamma$ -fructose. It forms a crystalline *amide*, m.p. 104-5°, and when methylated forms 2, 3, 4-*trimethoxy-d-erythronic acid methyl ester* (methyl trimethoxy-butyrate) which produces a crystalline *amide* m.p. 58-9° (37). A lactone of the dimethyl acid has been reported (11, 14).

2, 3-*Dimethyl-d-erythronolactone* is said to be obtained on allowing potassium permanganate to act on tetramethyl fructose.

## THREOSE

*Dimethoxy-d-succinic acid* has been variously reported as being prepared from l-trimethyl  $\gamma$ -arabonolactone (43), from tetramethyl  $\delta$ -gluconolactone and nitric acid (44), and from 2, 3, 5-trimethyl  $\gamma$ -xylonolactone (56). It forms a crystalline *amide* (43) and *methyl amide*, m.p.  $204.5^\circ$  (43, 56).

*Dimethoxy-l-succinic acid* is obtained from d-2, 3, 5-trimethyl  $\gamma$ -arabonolactone (43, 55). It forms a crystalline *amide* and *methyl amide* (43).

*Dimethoxy-l-succinic acid* results from the breaking down of the lactol acid of 1, 3, 4, 5-tetramethyl fructose (2). Its *amide* has a melting point of  $246^\circ$  with decomposition.

## ERYTHRULOSE

Tetramethyl  $\gamma$ -fructose, when oxidized with nitric acid, gave not a trimethoxyvalerolactone (25), but an ester believed to be the *methyl ester* of a *dimethyl-erythrulonic acid*. The compound was not characterized.

## PENTOSES

## RHAMNOSE

By cautious methylation of acetone rhamnose, *dimethyl acetone rhamnose* can be secured (18). Hydrolysis of this gives *dimethyl rhamnose* (9, 10). The *methyl rhamnoside* (3, 9) of this compound has  $[\alpha]_D^{95^\circ}$  ( $C_2H_5OH$ ) (3). This value, however, is approximate. *Dimethyl acetone rhamnoside* has  $[\alpha]_D = -31.1^\circ$  ( $CH_3OH$ ) and  $-35.32^\circ$  (*acetone*) (3).

Acetone methyl rhamnoside, when methylated, produces *trimethyl  $\alpha$ -methyl l-rhamnoside*,  $[\alpha]_D = -54.13^\circ$  (alc.) and  $-15.54^\circ$  ( $H_2O$ ) (3);  $-16^\circ$  ( $H_2O$ ) (30). When oxidized, this latter compound forms l-arabotrimethoxyglutaric acid (25). Again, when hydrolyzed, *trimethyl l-rhamnose* is obtained (1, 9, 13).

Trimethyl rhamnose is a colorless syrup, reducing Fehlings (3) and forming *trimethyl rhammonic acid* on oxidation (29). The trimethyl rhamnose,  $[\alpha]_D = +25^\circ$  ( $H_2O$ —final) (30), and, according to (3) has the values:  $[\alpha]_D + 24.15^\circ \rightarrow 25.44^\circ$  ( $H_2O$ );  $+3.25^\circ \rightarrow +5.82^\circ$  (benzene); and  $-4.86^\circ \rightarrow -9.52^\circ$  (alc). An *anilide*, is reported (5) as needles, m.p.  $111.3^\circ$ , and also a crystalline *phenylhydrazone* (3) decomposing at  $126.8^\circ$ . This hydrazone is slightly soluble in water, very soluble in alcohol, and moderately so in ether. When trimethyl rhamnose is treated with  $CH_3OH \cdot HCl$  a mixture of methyl rhamnosides is produced (3), largely the beta-form, showing  $+29.30^\circ$  ( $C_2H_5OH$ ) and  $+52.36^\circ$  ( $H_2O$ ).

The l-2, 3, 4-trimethyl rhamnose, when cautiously oxidized, produces a l-2, 3, 4-trimethyl  $\gamma$ -rhamnonolactone, b.p.  $120^\circ/0.3$  mm. and of m.p.  $40^\circ$  (70), which may be decomposed to l-trimethoxyglutaric acid. The rotation of the lactone is  $[\alpha]_D^{18} = -130^\circ$  (initial)  $\rightarrow -78^\circ$  (equil.). A *phenylhydrazide* may be obtained from this lactone, needles m.p.  $177^\circ$  (69). The trimethyl rhamnose already mentioned, which may also be obtained from l-2, 3, 4-trimethyl  $\beta$ -methyl rhamnopyranoside [m.p.  $43.4^\circ$ ;  $[\alpha]_D^{21} = +106^\circ$  ( $H_2O$ )], can also form (70) a 3, 4-dimethyl  $\delta$ -rhamnonolactone, long needles, m.p.  $66.8^\circ$ ,  $[\alpha]_D^{26} = -153^\circ$  (init.— $H_2O$ ), from which a 3, 4-dimethyl rhamnonamide, colorless needles, m.p.  $152.5^\circ$ , can be secured. The



amide is soluble in water, methyl or ethyl alcohols, but insoluble in light petroleum.

Gamma-monoacetyl  $\beta$ -methyl rhamnopyranoside, on methylation (70), forms a *2-monoacetyl-3, 4-di-methyl  $\beta$ -methyl rhamnoside* with the following characteristics: long, colorless needles, m.p.  $67^\circ$ . Very soluble in all solvents. B.p. about  $90^\circ/0.1$  mm.,  $N_D^{17} = 1.4510$  (supercooled liquid);  $[\alpha]_D^{20} = +36^\circ$  ( $H_2O$ ), and did not reduce Fehlings solution. On hydrolysis, a *3, 4-dimethyl rhamnose*, colorless needles, m.p.  $91-2^\circ$ ,  $[\alpha]_D^{20} = -18.6$  (equil.  $H_2O$ ) and  $[\alpha]_D^{20} = -10^\circ$  (initially). On oxidation this forms the *3, 4-dimethyl  $\delta$ -rhamnonolactone* mentioned above.

#### RIBOSE

d-Ribose, probably in the form of an alkyl compound, has been isolated from animal tissues (50).

#### LYXOSE

When  $\alpha$ -methyl lyxoside (m.p.  $109^\circ$ ) is methylated, *trimethyl methyl lyxoside* is obtained (54, 59), a colorless mobile oil, boiling point about  $70^\circ/0.02$  mm., and giving *d-trimethoxy glutaric acid* on treatment with  $HNO_3$ . It is a mixture, consisting largely of the  $\alpha$ -isomeride, has  $N_D^{14} = 1.4460$ , and  $[\alpha]_D^{20_{5461}} + 10^\circ$  ( $H_2O$ ) and  $+37.3^\circ$  ( $C_2H_5OH$ ). On removing the glycoside group, *2, 3, 4-trimethyl lyxose* is obtained (72).

*2, 3, 4-trimethyl lyxose* consists of needles, m.p.  $79^\circ$ ,  $[\alpha]^{20_{5461}} - 19^\circ$  (constant). Before crystallization, it is a colorless, mobile liquid, b.p.  $90^\circ/0.05$  mm.,  $N_D^{15} = 1.4629$ ,  $[\alpha]_D^{22} = -22^\circ$  ( $H_2O$ ). It exhibits rapid mutarotation in aqueous solution.

On oxidation, *trimethyl  $\delta$ -lyxonolactone* is formed, b.p.  $105^\circ/0.02$  mm.,  $N_D^{18} = 1.4620$ ,  $[\alpha]_D^{19} = +35.5^\circ$  (initial)  $\rightarrow -9.3$  (equil. in water). It forms a phenylhydrazide of m.p.  $180-1^\circ$  (59, 65). This lactone, as well as the *2, 3, 5-trimethyl  $\gamma$ -lyxonolactone*, is secured by the inversion of *2, 3, 4-trimethyl  $\delta$ -xylonolactone* and *2, 3, 5-trimethyl  $\gamma$ -xylonolactone* respectively in pyridine solution (65).

The  $\delta$ -lyxonolactone forms *d-trimethoxyglutaric acid* on oxidation (59, 73). This acid forms a *methyl ester* of b.p. about  $100^\circ/0.1$  mm.,  $n_D^{21} = 1.4353$ ,  $[\alpha]_D^{20} = -39^\circ$  ( $CH_3OH$ ) and  $-31^\circ$  ( $H_2O$ ). This methyl trimethoxy glutarate forms an amide, m.p.  $230^\circ$  (decomp'n);  $[\alpha]_D^{20} - 49^\circ$ . The acid itself forms a *methyl amide*, m.p.  $171-2^\circ$ .

Bott, Hirst and Smith (73) described the methylation of methyl-lyxofuranoside as follows: The product of the reaction, when distilled, came over as a colorless, hygroscopic liquid, b.p. about  $90^\circ/0.06$  mm.,  $N_D^{17} = 1.4457$ ,  $[\alpha]_D^{22} = +52^\circ$  ( $H_2O$ );  $[\alpha]_D^{22} = +41^\circ$  ( $CH_3OH$ );  $[\alpha]_D^{22} = +52^\circ$  (equilibrium value after heating with one per cent  $CH_3OH - HCl$ ). The presence of a small amount of trimethyl methyl lyxopyranoside was indicated. Since trimethyl lyxopyranose has  $[\alpha]_D^{20} = 22^\circ$  (equil.  $-H_2O$ ) and trimethyl lyxofuranose has  $[\alpha]_D^{22} = +39^\circ$  (equil.  $-H_2O$ ), it follows that the quantities of *trimethyl methyl lyxofuranoside* and *trimethyl methyl lyxopyranoside* in the distillate were about 90 per cent and 10 per cent, respectively. Hydrolysis of the distillate resulted in *2, 3, 4-trimethyl lyxofuranose*, b.p. about  $95^\circ/0.04$  mm.,  $N_D^{16} = 1.4580$ , and  $[\alpha]_D^{20} = +39^\circ$  ( $H_2O$ ).

On oxidation, this methylated sugar gave *trimethyl  $\gamma$ -lyxonolactone*, b.p.  $170^{\circ}/12$  mm., long needles, m.p.  $44^{\circ}$ ,  $N_D^{18} = 1.4569$ , and  $[\alpha]_D^{20} = +82.5^{\circ}$  (initial— $H_2O$ ). From this lactone, a *hydrazide*, needles, m.p.  $140^{\circ}$ , may be obtained.

When distilled slowly, the 2, 3, 4-trimethyl lyxofuranose forms a *hexamethyl di-pentose*, a colorless liquid, b.p. about  $160^{\circ}/0.05$  mm., which solidified to give needles, m.p.  $77^{\circ}$ . It has no action on Fehling's, but is strongly reducing after hydrolysis with hydrochloric acid.  $[\alpha]_D^{20} = +114^{\circ}$  (initial)  $\rightarrow +43^{\circ}$  (after 15 minutes hydrolysis).

#### ARABINOSE

##### *Dimethyl Arabinoses*

2, 4-Dimethyl arabinose is reported by Zemplen and Braun (36) as a syrup of b.p.  $128.9^{\circ}/0.32$  mm.,  $[\alpha]_D^{24} = 95.46^{\circ}$  ( $C_2H_5OH$ ) changing to  $-105.1^{\circ}$  after 24 hours. 2, 5-Dimethyl d-arabonolactone is secured (23) when 2, 3, 5, 6-tetramethyl glucose is treated with hydrogen peroxide. A dimethyl d-arabonolactone, not characterized, is reported (40) from the oxidation of 2, 3, 4, 6-tetramethyl glucose. An unidentified dimethyl arabinose, a glass, is secured from hexamethyl methyl maltoside (8) by hydrolysis. This dimethyl arabinose forms a methyl arabinoside of b.p.  $120^{\circ}/0.1$  mm., and this in turn produces on methylation, a trimethyl methyl arabinoside. A dimethoxy hydroxyvaleric acid (19) will be described under trimethyl arabinoses.

##### *Trimethyl Arabinoses*

2, 3, 4-Trimethyl l-arabinose [1, 5] (the normal form) is a colorless syrup (2) showing mutarotation, with a b.p. of  $148-152^{\circ}/19$  mm., very soluble in water, all organic solvents and light petroleum. When oxidized, 2, 3, 4-trimethyl arabonolactone [1, 5] is formed (25, 33, 46). This occurs in the form of long, colorless needles, m.p.  $45^{\circ}$  (38, 60),  $N_D^{15.5} = 1.4630$  (superfused) (60), and having an initial value in water at  $20^{\circ}$  of  $+179.5^{\circ}$  (38, 60);  $+176.5^{\circ}$  (49);  $+178.3^{\circ}$  (49). For other wave lengths  $[\alpha]_{5461} = +206.3^{\circ}$  ( $H_2O$ ) and  $[\alpha]_{215780}^{21} = +183.5^{\circ}$ . Mutarotation is recorded as follows:  $[\alpha]_D + 206^{\circ} \rightarrow +17^{\circ}$  (3 hours— $H_2O$ ) (38);  $+145^{\circ} \rightarrow 22.4^{\circ}$  (24 hours) (24). This lactone may also be prepared by methylation of arabonolactone (25). The trimethyl lactone, when oxidized with  $HNO_3$ , form l-arabotrimethoxyglutaric acid (25, 41, 46) which may also be prepared directly from the trimethyl l-arabinose [1, 5] (22, 31, 33, 41). This glutaric acid forms a crystalline amide (41, 46), decomposing at  $230^{\circ}$  with  $[\alpha] + 50^{\circ}$  ( $H_2O$ ) (41); a methyl amide, m.p.  $173^{\circ}$  (41),  $172^{\circ}$  (46) and with  $[\alpha]_D^{19} + 59.9^{\circ}$  (41, 46); a diamide (22, 33); a methyl ester (22, 46) and a dimethyl ester (32, 33).

2, 3, 4-trimethyl arabinose [1, 5] has been condensed with methyl chloroformate and KCN to give trimethyl arabomethoxymannonitrile and gluconitrile (66). An attempt (5) to prepare an anilide of the trimethyl arabinose resulted in a syrup. It was prepared (2, 9, 22, 32) from 2, 3, 4-trimethyl  $\alpha$ -methyl l-arabinoside [1, 5], m.p.  $43.5^{\circ}$  (2, 32);  $44.6^{\circ}$  (49), having  $[\alpha]_D = +250.8^{\circ}$  ( $H_2O$ ) (2, 49);  $+223.1^{\circ}$  ( $CH_3OH$ ) (49). This arabinoside is rapidly destroyed by fuming  $HCl$ . A l-2, 3, 4-trimethyl

$\beta$ -methyl arabinoside [1, 5] has been prepared (49). 2, 3, 4-Trimethyl  $\alpha$ -methyl arabinoside [1, 5] (32) forms a 2, 3, 4-trimethyl d-arabinose [1, 5] (72).

When 3, 4, 6-trimethyl  $\gamma$ -fructose (55) and 1, 3, 4, 6-tetramethyl fructose are degraded (37, 43, 53, 55, 64), d-2, 3, 5-trimethyl  $\gamma$ -arabonolactone [1, 4] is formed.

This is a pale yellow mobile syrup of b.p. 100-15°/0.12 mm., (55) and showing a melting point of 31.2° (55); 33° (43). In water,  $[\alpha]_D = +44.5^\circ \rightarrow +25.5^\circ$  (20 days) (43). On oxidation with  $\text{HNO}_3$  l-dimethoxy succinic acid is formed (37, 43, 53, 55, 65). This produces a crystalline amide and methyl amide.

On methylation,  $\gamma$ -methyl arabinoside,  $[\alpha]_D - 71.30^\circ$  ( $\text{CH}_3\text{OH}$ ), forms a mixture of  $\alpha$ - and  $\beta$ -trimethyl  $\gamma$ -methyl arabinosides (19), which exhibits  $[\alpha]_D = -55.8^\circ$  ( $\text{H}_2\text{O}$ ) (31);  $-56^\circ$  (equil.) (49). On hydrolysis (19), l-2, 3, 5-trimethyl  $\gamma$ -arabinose [1, 4] is formed. This is a colorless liquid,  $N_D = 1.4503$ , b.p. 97.9°/0.18 mm., reducing neutral permanganate and showing  $[\alpha]_D = 39.5^\circ$  ( $\text{H}_2\text{O}$ ) (19). When oxidized with  $\text{HNO}_3$  (19), a lactone of a trimethoxyhydroxyvaleric acid is first formed, which in turn forms a dimethyl trimethoxy-glutarate. l-2, 3, 5-Trimethyl  $\gamma$ -arabinose [1, 4], also forms on milder oxidation (27, 43) the l-2, 3, 5-trimethoxy- $\gamma$ -arabonolactone [1, 4], long, colorless needles, of melting point 29° (49), 30° (49), 30-32° (43), 32° (27) and 33° (37). It has an initial rotation in water of  $-42.5^\circ$  (49),  $-43^\circ$  (49),  $-44^\circ$  (43),  $-44.2^\circ$  (37) and  $-44.4^\circ$  (27, 38), which, after 20 days, is found to be  $-25^\circ$  (43),  $-25.1^\circ$  (24),  $-25.2^\circ$  (37, 38). This important lactone may also be secured by methylating  $\gamma$ -arabonolactone (25, 27, 49). On oxidation, the methylated lactone forms d-dimethoxysuccinic acid (43), which has likewise been reported as being obtained from trimethyl  $\gamma$ -xylonolactone (which see) and, among other degradation products from tetramethyl  $\delta$ -gluconolactone (44, 47). The succinic acid forms a crystalline amide (43) and a crystalline methyl amide (43) of melting point 204.5° (56).

Inactive dimethoxy succinic acid is reported (42) as one of the degradation products of oxidized 1, 3, 4, 5-tetramethyl fructose. 2, 3, 5-Trimethyl arabonic acid (37, 40) was expected to be the oxidative product of the 1, 2-ene diol form of 2, 3, 4, 6-tetramethyl glucose, but was not obtained. d-Arabotrimethoxy glutaric acid (67) may be prepared from the following:

- (a) 1, 3, 4, 5-tetramethyl fructose (25, 26, 42).
- (b) Trimethyl  $\delta$ -lyxonolactone (54, 59).
- (c) Trimethyl d-arabinose (32, 72).
- (d) Tetramethyl  $\delta$ -mannonolactone (39, 72).

The acid can be identified by (1) its amide (25, 26, 42, 59), of m.p. 227-8° (42), 230° (59) with decomposition, and  $[\alpha]_D^{20} = -48^\circ$  (42),  $-49^\circ$  (59) both in water; (2) by its methyl ester, b.p. about 100°/0.1 mm.,  $n_D^{21} = 1.4353$ ,  $[\alpha]_D^{20} = 39^\circ$  ( $\text{CH}_3\text{OH}$ ) and  $31^\circ$  ( $\text{H}_2\text{O}$ ) (59), and (3) by its dimethyl ester which is enantiomorphous with the dimethyl ester of the acid from trimethyl-l-arabinose.

A d-2, 3, 4-trimethyl  $\delta$ -arabonolactone [1, 5] is described (53, 60, 64) as being obtained from 1, 3, 4, 5-tetramethyl fructose, and recorded as long, colorless needles, m.p. 44°,  $N_D^{18} = 1.4626$  (superfused) and  $[\alpha]_D^{18}$  (initial)  $= -177.3^\circ$  ( $\text{H}_2\text{O}$ ).



Aceto-iodo-maltose was methylated (51, 80) and, instead of obtaining the desired heptamethyl methyl maltoside, a *hexamethyl methyl gluco-arabinoside* was obtained. Methylated arabinoses are mentioned, but not described (12, 13, 22, 29 and 52).

#### XYLOSE

##### *Monomethyl and Dimethyl Xyloses*

When monoacetone xylose is refluxed in acetone with  $\text{CH}_3\text{I}$  and  $\text{Ag}_2\text{O}$ , a mixture of *mono-* and *di-methyl acetone xyloses* is obtained (18, 54). The *monomethyl diacetone xylose* melted at  $78^\circ$ , boiled at  $105.7^\circ$  and  $[\alpha]_{\text{H}_2\text{O}}^{20} = 21.4^\circ$  ( $\text{H}_2\text{O}$ ). It gave very strong pentose reactions. When hydrolyzed with dilute sulfuric acid, the resulting equilibrium solution of *mono-methyl xylose* shows  $[\alpha]_{\text{D}} = 43.3^\circ$  ( $\text{H}_2\text{O}$ ). The *dimethyl acetone xylose* of b.p.  $78\text{--}80^\circ$  (18) is soluble in 6 parts water and  $[\alpha]_{\text{H}_2\text{O}} = -43.3^\circ$  (18),  $-46.6^\circ$  (56). According to Haworth and Porter (56), it is a syrup of b.p.  $75\text{--}80/0.07$  mm., and  $N_{\text{D}}^{15} = 1.4455$ .

When hydrolyzed, the dimethyl acetone xylose produces a *dimethyl methyl xylose* (7, 18, 54, 56) believed to be *3, 5-dimethyl xylose* (56). On oxidation, this forms a *3, 5-dimethyl  $\gamma$ -xylonolactone* (54, 56) of  $[\alpha]_{\text{D}}^{21.5}_{5780} = +39^\circ$  (49 days) (56) and showing  $N_{\text{D}}^{15} = 1.4643$ . The lactone can also be recognized from its crystalline *phenylhydrazide* (56), m.p.  $94\text{--}5^\circ$ , needles, soluble in  $\text{CHCl}_3$ , less so in ether or water, and insoluble in light petroleum.

When xylan is methylated, a dimethyl xylan is obtained (17, 63). On hydrolysis, *2, 3-dimethyl methyl xyloside* in a yield of 90 per cent is obtained, with characteristics of b.p.  $80^\circ/0.04$  mm.,  $N_{\text{D}}^{17} = 1.4581$ ,  $[\alpha]_{\text{D}}^{21.5} = +61.8^\circ$  ( $\text{CH}_3\text{OH}$ );  $[\alpha]_{\text{D}}^{22} = +43^\circ$  (equil. value). The xyloside produced *2, 3-dimethyl xylose*—viscid syrup, reducing Fehlings,  $N_{\text{D}}^{20} = 1.4783$ ,  $[\alpha]_{\text{D}}^{20} = +22.6^\circ$  ( $\text{H}_2\text{O}$ —initial)  $\rightarrow [\alpha]_{\text{D}}^{22} = +24^\circ$  (const.). Could not be crystallized. An *anilide* can be prepared of m.p.  $146^\circ$ ;  $[\alpha]_{\text{D}} = +65.5^\circ$  (ethyl acetate—constant);  $[\alpha]_{\text{D}}^{19} = +185^\circ$  (initial). The *2, 3-dimethyl xylose* can be oxidized to *2, 3-dimethyl  $\gamma$ -xylonolactone*—a colorless liquid—b.p.  $115^\circ/0.02$  mm.;  $N_{\text{D}}^{16.5} = 1.4640$ ;  $[\alpha]_{\text{D}}^{22} = +97^\circ$  (initial) ( $\text{H}_2\text{O}$ ). *2, 3-Dimethyl xylonic acid*,  $[\alpha]_{\text{D}}^{22} = +30.4^\circ$  ( $\text{H}_2\text{O}$ ) forms a *phenylhydrazide* of m.p.  $107\text{--}8^\circ$ , insol. in ether or light petroleum, but readily soluble in  $\text{H}_2\text{O}$ , alc., or ethyl acetate; and a *p-bromophenylhydrazide*, silky needles, m.p.  $150\text{--}1^\circ$  in a yield of 80 per cent (63).

When  $\beta$ -xylo-chloralose is methylated, *dimethyl  $\beta$ -xylochloralose*, b.p.  $135^\circ/2$  mm., forming oily crystals, m.p.  $53^\circ$ , is produced. When acetylated (74), a product is formed which is probably mostly *monoacetyl dimethyl  $\beta$ -xylochloralose*.

##### *Trimethyl Xyloses*

Trimethyl xyloses are mentioned but not described in the references (13, 20, 22 and 46). *2, 3, 4-Trimethyl methyl xyloside*, prepared from  $\beta$ -methyl xyloside (68) is a wax-like solid, m.p.  $46.8^\circ$  (9, 38) or  $51^\circ$  (68). In water,  $[\alpha]_{\text{D}}^{20} = -67^\circ$  (9),  $-81.7^\circ$  (68) and in  $\text{CHCl}_3$   $-69.5^\circ$  (68). Before crystallization, it is a syrup boiling at  $69\text{--}72^\circ/0.5$  mm., (9) or  $65^\circ/0.02$  mm., (63) with  $N_{\text{D}}^{18} = 1.4403$  (63). When the glycosidic methyl



group is removed, 1-2, 3, 4-trimethyl  $\delta$ -xylose [1, 5] is the result (9, 38, 68). This sugar has a melting point of 87-90° (28), 91-2° (68) and 90-3° (63). Mutarotation is quite rapid in aqueous solution:  $[\alpha]_D^{20} = +64.5^\circ$  (initial)  $\rightarrow +17.7^\circ$  (final) (68) or  $+74^\circ \rightarrow +21^\circ$  (28).

On oxidation (28, 69), 1-2, 3, 4-trimethyl  $\delta$ -xylonolactone [1, 5] is formed. This lactone melts at 55° (28), 56° (38), being colorless needles of  $[\alpha]_D \pm 0^\circ$  (H<sub>2</sub>O)  $\rightarrow +21.4^\circ$  (70, 75 hrs.) (38) or  $-3.8^\circ \rightarrow +20.8^\circ$  (final) (24, 28). In pyridine solution, a molecular change takes place, and the corresponding  $\delta$ -xylonolactone is produced. The xylonolactone, in common with the mother trimethyl xylose (16, 72), forms *i*-xylotrimethoxyglutaric acid (28, 46), which is described later.

2, 3, 4-Trimethyl  $\delta$ -xylose [1, 5], on oxidation, also gives 2, 3, 4-trimethyl  $\delta$ -xylonic acid (16). For the acid, calculated as the lactone,  $[\alpha]_D^{16.5461} = +32.7^\circ$  (H<sub>2</sub>O)  $\rightarrow +21.5^\circ$  (8 hrs.). The acid can be recognized (65) from its *phenylhydrazide*, needles melting at 137-8.5°.

2, 3, 4-Trimethyl  $\alpha$ -methyl xyloside (9, 15, 69) results from the methylation of  $\alpha$ -methyl xyloside, and when containing 34 per cent of the beta-isomeride is a mobile syrup, b.p. 115-8°/12 mm.,  $N_D^{25} = 1.4410$  and  $[\alpha]_D = 86^\circ$  (CH<sub>3</sub>OH) (9). When pure, it shows  $N_D^{25} = 1.4397$  and the following rotations:  $[\alpha]_D^{20} = +121.5^\circ$  (CHCl<sub>3</sub>);  $+112.7^\circ$  (H<sub>2</sub>O) and  $+122.2^\circ$  (CH<sub>3</sub>OH) (68).

*Inactive xylotrimethoxyglutaric acid*, a syrup, is the product resulting from the oxidative degradation of various methylated hexoses. Thus, it results when HNO<sub>3</sub> is allowed to act on 2, 3, 4, 6-tetramethyl  $\delta$ -gluconolactone (25, 29, 34, 35, 44, 53, 64, 66), on 2, 3, 4, 6-tetramethyl glucose (25, 35, 52, 66) or on tetramethyl  $\gamma$ -fructose (45). The acid forms a *methyl amide* (44, 46, 53, 64), m.p. 167-8° (46); an *amide* (45, 46), m.p. 101°,  $[\alpha]_D = -76^\circ$  (H<sub>2</sub>O) (45); a syrupy *dimethyl ester* (16) and a *crystalline diamide* (16). It also results from the oxidation of 2, 3, 4-trimethyl xylose [1, 5] (16) trimethyl  $\delta$ -xylonolactone (46), and 2, 3, 4-trimethyl  $\beta$ -methyl xyloside (16).

Gamma-methyl xyloside forms 2, 3, 5-trimethyl methyl xyloside [1, 4] (31), a colorless liquid boiling at 82.5-84.5°/0.03 mm., or 110-4°/14 mm., (28), with  $N_D = 1.4387$  and showing  $[\alpha]_D = +32.0^\circ$  (CH<sub>3</sub>OH). On hydrolysis, 2, 3, 5-trimethyl  $\gamma$ -xylose is formed (28, 38). This is a liquid containing both  $\alpha$ - and  $\beta$ -forms, b.p. 110°/0.04 mm., (28), reducing alkaline permanganate and Fehlings solution. It combines rapidly with acid CH<sub>3</sub>OH.  $[\alpha]_D = +24.7^\circ \rightarrow +31.2^\circ$  (H<sub>2</sub>O).

This trimethyl xylose, in turn, oxidizes to 2, 3, 5-trimethyl  $\gamma$ -xylonolactone (28, 38, 56), which also results by methylation (56). The trimethyl xylonolactone is a mobile, colorless liquid boiling at 82°/0.06 mm., (56),  $N_D^{17} = 1.4464$  (56);  $N_D^{20} = 1.4426$  (63). It shows  $[\alpha]_D = +74.1^\circ$  (H<sub>2</sub>O)  $\rightarrow +61.4^\circ$  (504 hrs.) (24, 28, 38) and  $[\alpha]_D^{16.5461} = +100^\circ$  (initial) (H<sub>2</sub>O) (63). The lactone forms a crystalline *phenylhydrazide* (38, 56), m.p. 89-90° (56, 63), changes 2, 3, 5-trimethyl  $\gamma$ -xylonolactone in pyridine (65) and oxidizes to d-dimethoxy-succinic acid (56) already described (see under arabinose).

#### GENERAL

Saccharin ( $\gamma$ -lactone of 2-methyl-2, 3, 4, 5-tetrahydroxypentanoic acid) is reported (75) to give a 2-methyl pentose, an aldose, l-rotatory, producing

a violet color with alpha-naphthol and sulfuric acid, and not yielding methyl furfural nor an osazone.

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# THE LITERATURE OF ALKYLATED CARBOHYDRATES

## II. GALACTOSE DERIVATIVES

HAROLD W. COLES

*From the Laboratory of Plant Chemistry, Chemistry Department, Iowa State College,  
and Lilly Research Laboratories, Indianapolis, Indiana*

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### INTRODUCTION

The literature on alkylated galactose is extensive, hence the limitations imposed in the first paper of this series also hold in this paper. Methylated galactose is mentioned but not specifically described in many papers (5, 11, 13, 14, 16, 18, 25, 27, 39, 40, 41 and 47).

### MONOMETHYL GALACTOSE

Little is mentioned about monomethyl galactose derivatives. Freudenberg (34) reports that *diacetone-6-methyl galactose*, having a boiling point of 109°-15° at 0.2-0.5 mm., and said to be a thick uncrystallizable liquid (15) with  $[\alpha]^{19}_{\text{Hg}}$  (Yellow) = -63.2° (acetylene tetrachloride), forms *6-methyl galactose* on hydrolysis and this, in turn, produces a *monomethyl galactonic acid* and an *osazone*. The *6-methyl galactose* is also mentioned by Freudenberg (56). Dimethyl galactose is said to give a *monomethyl galactosazone* (29).

### DIMETHYL GALACTOSE

The only mention of a dimethyl galactose is that of Irvine and Oldham (29), who state that *dimethyl galactose* forms a monomethyl galactosazone.

### TRIMETHYL GALACTOSE

Alpha methyl galactoside, when methylated, forms a *trimethyl alpha-methyl galactoside* (7, 8).

d-Galactose, when methylated and oxidized, forms 2, 3, 4-trimethoxy l-glutaric acid.

### TETRAMETHYL GALACTOSE

It is reported that methylated dihydrosphingosine (coming from the partial cleavage of cerebrosides) gave (53) tetramethyl galactose, which was not isolated.

### 2, 3, 4, 6-Tetramethyl $\delta$ -galactose

This well known sugar is mentioned in (28) and (36) and is said to result (38) when the methylated sugar residue of the cerebrosides of ox-brain are hydrolyzed, a *tetramethyl methyl galactoside* first being produced.

The tetramethyl sugar may be obtained from the following sources:

- (1) Octamethyl lactose (10, 32, 44, 51, 57).
- (2) Octamethyl melibiose (45).
- (3) Hendecamethyl raffinose (17, 18, 42, 49).
- (4) Methyl octamethyl lactobionate (46).
- (5) Tetramethyl methyl galactoside (38).

The characteristics of this amylene oxide tetramethyl galactose have been reported in the literature as follows:

$[\alpha]_D$		M.P.	B.P.	Ref.
+ 117°	(equil)	71-2°		(44, 45)
+ 109°	(CHCl <sub>3</sub> )			(50)
+ 109.7°			136°-40°/1 mm.	(37)
+ 149.4°		71.3°		(42)
+ 115°				(38)
+ 109.5°	(water)		172°/13 mm.	(2)
+ 62.5°	(Alc.)			(2)
+ 90.0°	(benzene)			(2)

It is said (2) to be soluble in water, alcohol, ether, benzene, and with difficulty soluble in light petroleum. It reduces Fehlings on warming.

2, 3, 4, 6-Tetramethyl galactose forms a characteristic *tetramethyl galactose anilide* (10, 17, 18, 22, 42, 44, 45) which has a melting point of 192°-3° (6, 42, 45), shows a rotation of  $[\alpha]_D = +38.0^\circ$  (equil.) (10), and is with difficulty soluble in water (6, 10, 12). This insolubility is of practicable use in the separation of the tetramethyl galactose (12) from 2, 3, 6-trimethyl glucose, since the anilide of the glucose derivative is rather soluble in water. The tetramethyl galactose, however, forms an oil with phenylhydrazine (2).

The oxidation products of 2, 3, 4, 6-tetramethyl galactose are also well-known. *Tetramethyl  $\delta$ -galactonic acid* may be obtained (30), which has a rotation, calculated as the lactone, of  $[\alpha]_D^{21} = +24^\circ \rightarrow +26.3^\circ$  (H<sub>2</sub>O-48 hrs.) (43). 2, 3, 4, 6-Tetramethyl  $\delta$ -galactonolactone (24) may also be prepared by oxidation of the parent sugar (21, 35, 43, 44, 55) or from the 2, 3, 4, 6-tetramethyl galactonic acid phenylhydrazide, m.p. 135-7° (44). When the rotation of the lactone has been measured, the following figures have been obtained:  $[\alpha]_D = +106.7^\circ \rightarrow +16.7^\circ$  (21);  $[\alpha]_{5461} = +191^\circ$  (43);  $[\alpha]_D = +161.5^\circ \rightarrow +27.2^\circ$  (24 hrs.) (44). It is said (44) to have a boiling point of 163-6°/0.18 mm., and a refractive index at 14° of 1.4606.

When the methylated lactone is oxidized with nitric acid (1.42), the following results (44):

- (1) No tetramethoxy mucic acid.
- (2) Trace of d-dimethoxysuccinic acid (diamide).
- (3) Methyl l-arabotrimethoxy glutarate.
  - (a) Methyl amide—m.p. 173°;  $[\alpha]_D^{19} = +59.9^\circ$ .
  - (b) Amide—m.p. 230° (decomp'n);  $[\alpha]_D = +50^\circ$  (H<sub>2</sub>O).

Conductivity measurements have been made on this lactone (58).

### 2, 3, 5, 6-Tetramethyl $\gamma$ -galactose

When heptamethyl  $\beta$ -methyl lactoside is hydrolyzed, 2, 3, 5, 6-tetramethyl  $\beta$ -methyl galactoside (39) is formed. This can be separated from

the 2, 3, 6-trimethyl methyl glucoside by changing it into the *benzoyl compound* (22). The following physical constants have been reported for this galactoside:

$[\alpha]_D$		M.P.	B.P.	Ref.
+ 19.59°	(H <sub>2</sub> O)	48-48.5°		(22)
+ 30.7°	(H <sub>2</sub> O)	44.5°		(2)
			87°/0.035 mm.	(22)
+ 30.7°	(H <sub>2</sub> O)			(4)
— 20.9°	(alc.)			(4)
+ 20.7°	(H <sub>2</sub> O)			(54)
— 24.42°	(alc.)			(54)
— 20.9°	(alc.)	44.5°		(1)
+ 30.7°	(H <sub>2</sub> O)			(1)
— 45.2°	(H <sub>2</sub> O)			(24)

This galactoside, which has a refractive index of 1.4420 (22), may also be obtained if  $\beta$ -methyl galactoside is methylated (3, 9). When hydrolyzed, 2, 3, 5, 6-tetramethyl  $\gamma$ -galactose (20) is produced (9, 22). This sugar undergoes a spontaneous auto-condensation to a non-reducing disaccharide (9, 19, 26). It is a colorless liquid reducing neutral permanganate (24), boiling at 136°/0.05 mm., having  $[\alpha]_D = -21.2^\circ$  (H<sub>2</sub>O-equil.), and  $N_D = 1.454$ .

When the tetramethyl  $\gamma$ -galactose is oxidized, the well-characterized 2, 3, 5, 6-tetramethyl  $\gamma$ -galactono-lactone [1, 4] is produced (35, 43). It may also be prepared by methylating galactono-lactone (21, 52, 55), or from the tetramethyl  $\gamma$ -galactonic acid,  $[\alpha]_{5461} = -8.3^\circ$  (24). The rotation of this methylated lactone is variously stated to be  $-29.5^\circ \rightarrow -27.0^\circ$  (21);  $-27.1^\circ \rightarrow -25.2^\circ$  (H<sub>2</sub>O—20 days) (31);  $-27^\circ$  (H<sub>2</sub>O)  $\rightarrow -25^\circ$  (12 days) (43);  $-27.1^\circ \rightarrow -25.2^\circ$  (H<sub>2</sub>O) (24);  $[\alpha]_{5461} = -31^\circ$  (43). Conductivity measurements for this lactone have been published (58).

Alpha-methyl galactoside, when methylated, forms tetramethyl  $\alpha$ -methyl galactoside (1, 2, 7, 8). This galactoside (39) is described as follows:

$[\alpha]_D$		B. P.	Ref.
+ 143.4°	(H <sub>2</sub> O)		(4)
+ 109.9°	(alc.)		(4)
+ 143.4°	(H <sub>2</sub> O)	88-90°/1 mm.	(37)
+ 188.5°	(H <sub>2</sub> O)		(54)
+ 148.0°	(alc.)	80.4°/0.05 mm.	(54)
+ 105.7°	(alc.)		(1, 2)
+ 143.4°	(H <sub>2</sub> O)	136-7°/11 mm.	(1, 2)

It is readily soluble in organic solvents.

#### PENTAMETHYL GALACTOSE

A liquid aldehydo pentamethyl galactose has been described (48, 59). This pentamethyl galactose, of  $[\alpha]_D^{20} = -4.8^\circ$  (48) readily condenses with alcohol in the absence of catalyst to form a dimethyl acetal, showing

$[\alpha]_D^{20} = 0$ . The acetal is readily hydrolyzed, reducing alkaline permanganate and ammoniacal silver solution. It has a b.p. of  $118-20^\circ$  at 0.6 mm., and also shows no appreciable rotation in acetylene tetrachloride. The pentamethyl galactose shows in methyl alcohol:  $[\alpha]_D = 0^\circ$  (initial)  $\rightarrow -6^\circ$  (1 hr.)  $\rightarrow -10^\circ$  (const.). When HCl is added to the alcohol solution, the rotation immediately becomes  $-10^\circ$ .

Levene and Meyer (48) also describe a *pentamethyl diethylmercapto galactose*—b.p.  $155-160^\circ/0.2$  mm. In a concentration of 11 per cent, it does not show any rotation in methyl alcohol.

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# THE LITERATURE OF ALKYLATED CARBOHYDRATES

## III. ALKYLATED MANNOSE DERIVATIVES

HAROLD W. COLES

*From the Laboratory of Plant Chemistry, Chemistry Department, Iowa State College,  
and Lilly Research Laboratories, Indianapolis, Indiana*

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### INTRODUCTION

Methylated derivatives have been widely employed in a study of the structural configurations possible in the case of the important sugar, mannose. Consequently, the references to alkylated derivatives are many. The same limitations apply in this paper as given in the first of the series.

### MONOMETHYL MANNOSE

When diacetone mannose is methylated, *diacetone methyl mannose* is formed (16, 23, 25, 36, 45). This has a boiling point of 111.5° (45); 118.24°/0.2-0.5 mm. (16), is a colorless liquid of glycerin consistency and, when crystallized, has a melting point of 37° (16). It shows with the yellow band of Hg,  $[\alpha]^{19} = -41.0^\circ$  (16, 36);  $-42.2^\circ$ . On hydrolysis, it does not yield a methylated mannose as expected, but pure mannose (16, 23). A *methyl diacetone mannose*, an isomer of the above, is reported (23),  $[\alpha]_D = +23^\circ$  in acetylene tetrachloride, b.p. 115°/1.2 mm., also giving alpha-mannose on hydrolysis, but it is declared to be a glycoside (36). Methyl mannoside produces a *methyl diacetone mannose* (23) of b.p. 105°/0.5 mm., with  $[\alpha]_D^{20} = +34.9^\circ$ .

### DIMETHYL MANNOSE

A *dimethyl mannose* is reported as being obtained, along with other methylated mannoses, from vegetable ivory (19). When gamma-mannonolactone is methylated and treated with acetone (42), a *dimethyl  $\gamma$ -mannonolactone monoacetone* is formed. This has a melting point of 109-110°;  $[\alpha]_D^{20} = +64.2^\circ$  (H<sub>2</sub>O)  $\rightarrow +55.8^\circ$  (9 days). When a partial hydrolysis is carried out, *dimethyl  $\gamma$ -mannonolactone* is produced. This is said to have a melting point of 109°-110°;  $[\alpha]_D^{20} = +61.1^\circ$  (H<sub>2</sub>O)  $\rightarrow 60.5^\circ$  (19 days).

Diacetone mannitol, on methylation, forms a *5, 6-dimethyl mannitol diacetone* (5, 8), a colorless, mobile liquid of b.p. 140-1°/13 mm. Its optical rotation is  $[\alpha]_D^{20} = +21.9^\circ$  (alc.). Removal of the acetone residue leaves *5, 6-dimethyl mannitol* (5, 8),  $[\alpha]_D^{20} = -8.8^\circ$  (alc.) (5, 9), and this in turn, on oxidation, leads to *5, 6-dimethyl mannonic acid*, characterized by a *Ca salt*. A *5, 6-dimethyl mannonolactone* may be prepared from this acid (8).

## TRIMETHYL MANNOSE

A number of unusual trimethyl derivatives are mentioned. Thus, normal arabo-pyranose has been condensed with methyl chloroformate and KCN to obtain (54) a crystalline *trimethyl carbomethoxy mannonitrile*, from which a *1-2-carbomethoxy-3, 4, 6-trimethyl mannonic acid* of melting point  $155^\circ$  may be obtained. The same authors (54) mentioned a *l-3, 4, 6-trimethyl mannonolactone*, m.p.  $96-7^\circ$ , prisms, showing a constant rotation after 74 hours of  $+112.8^\circ$ . When  $\alpha$ -methyl mannoside is methylated, a *trimethyl methyl mannoside*, b.p.  $150^\circ/0.12$  mm., is formed along with tetramethyl mannoside (7, 10). The same tetramethyl mannoside is obtained from *trimethyl  $\gamma$ -mannoside* (48). Vegetable ivory is said (19) to yield a *trimethyl mannose* along with other methylated mannoses.

The following steps have been carried out (57): *Gamma-monoacetyl methyl mannoside*  $\rightarrow$  *monoacetyl trimethyl methyl mannoside*, a colorless liquid, b.p.  $120^\circ/0.1$  mm.;  $N_D^{15} = 1.4594$ ;  $[\alpha]_D^{23} + -20^\circ$  ( $H_2O$ );  $-11^\circ$  ( $CHCl_3$ )  $\rightarrow$  *d-3, 4, 6-trimethyl  $\alpha$ -mannopyranose*. This sugar shows a melting point of  $101.2^\circ$ ;  $[\alpha]_D^{22} = +36^\circ$  ( $CH_3OH$ );  $+21^\circ$  ( $H_2O$ );  $+19^\circ$  (3 mins.). Mutarotation takes place in water at  $22^\circ$ . When oxidized, the trimethyl sugar yields a *d-3, 4, 6-trimethyl  $\delta$ -mannonolactone* of m.p.  $96-7^\circ$ ;  $[\alpha]_D^{19} = +167.5^\circ$  ( $H_2O$ ), and this, in turn, a *d-3, 4, 6-trimethyl mannonic acid phenylhydrazide* of melting point  $137-9^\circ$ .

## TETRAMETHYL MANNOSE

The tetramethyl derivatives of mannose are well-known, and make up most of the alkylated derivatives. A tetramethyl mannose is said (19) to be obtained from the methylated vegetable ivory. Tetramethyl mannose is mentioned in the references 1, 12, 27, 30, 37, 55, 60, 61, 63, 64, 62.

*Gamma or Mannofuranose Types*

The gamma tetramethyl mannose is mentioned, but not specifically described, in 17, 18, 20, 21, 26, 32, 39, 40 and 43. The *d-2, 3, 4, 6-tetramethyl  $\gamma$ -mannose* is said to be obtained from gamma-methyl mannoside (8, 22, 33, 65); from  $\alpha$ -tetramethyl  $\gamma$ -methylmannoside (22); and is present, but as an impurity, in *2, 3, 5, 6-tetramethyl mannitol* (5), although the mannitol derivative can be made (8) from the tetramethyl mannose. The  $\alpha$ -tetramethyl  $\gamma$ -methyl mannoside is described as follows:

B.P.	mm.	$[\alpha]_D$	Solvent	Ref.
108-10°	0.1			(7, 10)
90°	0.05			(59)
105°	0.3	+ 24.7	water	(48)
120°	0.23	+ 98.6	"	(59)
		+ 65	"	(59)

It is also a colorless oil, of  $N_D^{16} = 1.4441$  (59).

The physical constants of the *d-2, 3, 5, 6-tetramethyl  $\gamma$ -mannose* are also described variously:



M.P.	B.P.	Pressure	$[\alpha]_D$	Solvent	Ref.
50-1°			+ 27.5°	CH <sub>3</sub> OH	(50)
			+ 48.5°	water	(34)
50-1°			+ 2.4°	"	(46)
			+ 27.6°	CH <sub>3</sub> OH	(46)
50-1°			+ 23.0°	CHCl <sub>3</sub>	(46)
	190°	10 mm.	+ 48.5°	CH <sub>3</sub> OH	(22)
	124°	0.1 mm.	+ 43.0°	water	(59)
			+ 37.0°	CH <sub>3</sub> OH	(59)

In liquid form, it is colorless,  $N_D^{15} = 1.4532$  (59), and is able to reduce  $KMnO_4$  vigorously at 15° (22). It crystallizes in the form of prism clusters (22). On oxidation (24, 33, 41, 46, 48, 65), is formed the highly important *d*-2, 3, 5, 6-tetramethyl  $\gamma$ -mannonolactone (37, 58), which may also be obtained from gamma-mannonolactone (42, 48, 49) or by the conversion of the corresponding gluconolactone (53). Physical constants of this lactone are found in the following table:

M.P.	B.P.	Pressure	$[\alpha]_D$	Solvent	Ref.
107°			+ 66.6°	water	(49)
			+ 64.0°	" (6 hrs.)	(49)
			+ 63.0°	" (24 hrs.)	(49)
	174°	11 mm.	+ 78.8°	dil. alc.	(5, 15)
			- 22.5°	" "	(5, 15)
106-7°			+ 63°	water	(41)
			+ 61°	" (9 days)	(41)
108°			+ 65°	" (Initial)	(59)
			+ 65° $\rightarrow$ 56.3°	" (20 days)	(31)
108°			+ 65.2° $\rightarrow$ 61.2°	" (9 days)	(42)
109°					(53)
107-8°			+ 64.8°		(48)

Crystalline form may be either needles (53) or colorless plates (49). Catalysis with HCl gave an equil. rotation of + 53° (41), and  $[\alpha]_{5461}^{18} = + 77^\circ \rightarrow + 71.8^\circ$  (9 days) (42). The rates of hydrolysis of this lactone have been calculated (47, 52). On destructive treatment, the lactone breaks down to inactive-dimethoxy-succinic acid (42), which is identified by a methyl ester and an amide. This lactone also forms a crystalline *phenylhydrazide* (53), of melting point 167° (53).

Other derivatives prepared from the tetramethyl  $\gamma$ -mannose are a *tetramethyl di-mannose* (22), indicating auto-condensation; an *anilide* (4, 5, 46, 50), with a variable melting point, and rotation of - 8° (CH<sub>3</sub>OH) (50) and - 8.5° (C<sub>2</sub>H<sub>5</sub>OH-final) (46); and a *d*-2, 3, 5, 6-tetramethyl *mannonic acid* (5, 8, 15, 28, 29, 44), of  $[\alpha]_D^{21} = - 23^\circ$  (H<sub>2</sub>O)  $\rightarrow - 17^\circ$  (15 days) (41), which forms a 5-ring lactone only (38, 41).

A *l*-isomeride of the  $\gamma$ -lactone is also described (49). This *l*-2, 3, 5, 6-tetramethyl  $\gamma$ -mannonolactone is described as long, narrow, colorless plates, m.p. 109°. They are readily soluble in ether and alcohol, less soluble in

water. Optical activity is shown by  $-65.51^\circ$  (6 mins.);  $-64.11^\circ$  (34 hrs.);  $-60.6^\circ$  (5 days);  $-47.4^\circ$  (18 days).

### Normal or Mannopyranose Types

2, 3, 4, 6-Tetramethyl  $\alpha$ -methyl mannoside (7, 10, 39) has been variously described. It is said to have a melting point of  $37-8^\circ$  (2),  $38-40^\circ$  (41) and  $39-40^\circ$  (46). It is, before crystallization, a limpid, colorless liquid, unaffected by emulsin (2) and with a boiling point of  $105^\circ/0.03$  mm. (41) and  $116^\circ/2$  mm. (46). Its rotation in water is  $+43^\circ$  (35);  $+42.9^\circ$  (2) and  $+43.5^\circ$  (46). Other rotations (see also 3) are  $+70.5^\circ$  ( $\text{CH}_3\text{OH}$ ) and  $+75.5^\circ$  (alc.) (2). Its refraction has been calculated as  $N_D^{16} = 1.4494$ .

Its beta-isomeride, 2, 3, 4, 6-tetramethyl  $\beta$ -methyl mannoside, when mixed with 12 per cent of the alpha, shows b.p.  $90^\circ/0.04$  mm.,  $N_D^{19} = 1.4521$  and  $[\alpha]_D = -65^\circ$  (water) (57). When crystallized, needles of melting point  $36-7^\circ$  are formed, having  $[\alpha]_D^{24} = -78^\circ$  ( $\text{H}_2\text{O}$ ). Partial hydrolysis, with removal of the glycoside group, forms *d*-2, 3, 4, 6-tetramethyl  $\delta$ -mannose (2, 35, 41 and 46), which may also be obtained from  $\alpha$ -methyl mannoside (22, 33, 42, 65) without first separating the methylated mannoside. The tetramethyl mannose has been given the following constants:

Nature	B.P.	Pressure	Rotation	Ref.
Syrup	114.5°	0.35 mm.	$+26^\circ \rightarrow +31^\circ$ ( $\text{CH}_3\text{OH}$ )	(41)
Liquid			$+17.2^\circ$ ( $\text{CH}_3\text{OH}$ —equil.)	(34)
			$+1^\circ$ ( $\text{H}_2\text{O}$ )	(35)
Viscid syrup	187-9°	19 mm.		(2)
Syrup	187°	19 mm.	$+17.2^\circ$ ( $\text{CH}_3\text{OH}$ )	(22)
			$+45.6^\circ$ ( $\text{C}_2\text{H}_5\text{I}$ )	(3)

It shows  $N_D^{19} = 1.4597$ , causes no reduction of potassium permanganate and reacts but slowly with acid methyl alcohol at  $100^\circ$  (22). It is said (46) to give some methylated glucose on vacuum distillation.

On oxidation the tetramethyl mannose forms 2, 3, 4, 6-tetramethyl *mannonic acid* (28, 29) and *d*-2, 3, 4, 6-tetramethyl  $\delta$ -mannonolactone, the normal lactone (24, 37, 41, 42, 51, 46, 33, 58, 65). This lactone has also been obtained by the conversion (53) of the corresponding normal gluconolactone. Rates of hydrolysis of the lactone have been calculated (47, 52). It is broken up, on oxidation, with formation of *d*-arabo-trimethoxy glutaric acid (56).

The physical characteristics of this lactone, which is a colorless syrup, before purification and crystallization, are stated to be the following:

M.P.	B.P.	Rotation	Ref.
	117°/0.04 mm.	$[\alpha]^{19}_{5461} = +156^\circ$ ( $\text{H}_2\text{O}$ )	(41)
23-4°	104°/0.02 mm.	$[\alpha]^{18}_{5780} = +153^\circ$ ( $\text{H}_2\text{O}$ )	(41, 42)
		$+172^\circ \rightarrow +73.4^\circ$ (146 hrs.)	(41, 42)
		$[\alpha]^{18}_{5461} = +172.3^\circ$	(41, 42)
		$+150^\circ$ ( $\text{H}_2\text{O}$ ) (18°)	(41, 42)
		$+105 \rightarrow +45.6^\circ$ (24 hrs.)	(31)
		$+136.4^\circ \rightarrow +62.4^\circ$ (final)	
		$+132.3^\circ \rightarrow +63.6^\circ$ (9 days)	(49)

Index of refraction is said to be  $N_D^{17} = 1.4643$  (41) and  $N_D^{16} = 1.4650$  (42). The 1-2, 3, 4, 6-tetramethyl  $\delta$ -mannonolactone (54) has a b.p. of 145-50°/0.06 mm.,  $[\alpha]_D^{18} = -150^\circ$  (15 mins.);  $-95.6^\circ$  (28 hrs.);  $-69.4^\circ$  (98 hrs.), and  $-58.2^\circ$  (15 hrs.—const.).

The normal form, the d-lactone, may be identified by the d-2, 3, 4, 6-tetramethyl  $\delta$ -mannonolactone phenylhydrazide (41, 42), white, nacreous flakes melting at 184-5° (41, 53); 183.4° (54). Rotation is  $[\alpha]_D^{16} = -22^\circ$  (54). Its structure has been discussed by Levene and Simms (38). The corresponding l-phenylhydrazide is claimed to have a melting point of 183-4° and  $[\alpha]_D = +22^\circ$  (54).

#### *Tetramethyl mannitols*

When mannitol monoacetone is methylated (5, 8, 14, 15), 3, 4, 5, 6-tetramethyl mannitol monoacetone is formed. This shows a b.p. of 137°-140°/11 mm., and a rotation at 20° of  $+32.2^\circ$  (5). When the acetone residue is removed from this compound, 3, 4, 5, 6-tetramethyl mannitol is formed (5, 8, 14, 15). This methylated mannitol (6, 11) boils at 167-9°/13 mm. (5), and exhibits an optical rotation of  $-12.5^\circ$  (5, 9). On oxidation (8), 3, 4, 5, 6-tetramethyl mannonic acid is formed, b.p. 180-2°/12 mm.,  $[\alpha]_D^{20} = +10.13$  (5), which is said to give no lactone (5, 8). 3, 4, 5, 6-tetramethyl  $\beta$ -ethyl mannitol is also described (90). The effect of boric acid on the conductivity and specific rotation of methylated mannitol has been published. 1, 2, 3, 4-tetramethyl-5-ethyl mannitol is said (14, 15) to be stable toward permanganate in the presence of NaOH in spite of the free terminal hydroxyl group.

#### PENTAMETHYL MANNOSE

2, 3, 4, 5, 6-Pentamethyl mannitol is stated to have a rotation, using sodium light, of  $+9.8^\circ$  (9).

When diethyl mercaptomannose is methylated (44), pentamethyl diethyl mercaptomannose is produced. This boils at 155-160°/0.2 mm., rotates at 20° of  $+39.4^\circ$  (methyl alcohol) and when the diethyl mercapto group is removed, gives rise to pentamethyl mannose. This compound boils at 98-100°/0.1 mm., reduces Fehling's on warming and alkaline silver nitrate in the cold. Optical rotation is given at  $+9.1^\circ$  (acetylene tetrachloride). In cold methyl alcohol (5°), initially the rotation is  $+8^\circ \rightarrow +12.30^\circ$  (10 mins.)  $\rightarrow +17.8^\circ$  (equil.). Pentamethyl mannose readily condenses with alcohol in the absence of catalyst to form pentamethyl mannose dimethyl acetal of boiling point 112-4°/0.1 mm.,  $[\alpha]_D^{20} = +21.2^\circ$  (CH<sub>3</sub>OH) and  $+19.3^\circ$  (acetylene tetrachloride).

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# ORIENTATION IN THE FURAN NUCLEUS<sup>1</sup>

HENRY GILMAN AND GEORGE F. WRIGHT

*From the Chemical Laboratory of Iowa State College*

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## INTRODUCTION

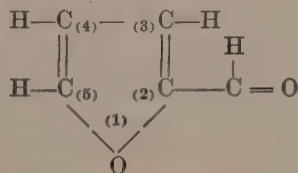
Furan compounds, of which furfural is the most important type, have properties which warrant their formal classification with aromatic types like benzene, thiophene and pyrrole. The most interesting and most valuable derivatives of an aromatic type like benzene are the parent compounds derived by direct nuclear substitution reactions such as halogenation, nitration, sulfonation and mercuration. Until recently such important types derivable by direct nuclear substitution of the very important furfural have not been prepared. They are now becoming available<sup>2</sup> and are being studied primarily with a view to their technical application.

The intelligent utilization of these new compounds rests fundamentally on an exact knowledge of their constitution. There are two general methods for determining the structure or the position of substituents in the nucleus. The first of these is a relative method which refers the unknown compound to known compounds. The second is a so-called absolute method and depends on the mathematical number of isomeric derivatives which might be obtained by successive substitution reactions.

The relative method with furan compounds is drastically limited for two reasons: first, there are but few reference compounds; and, second, even some of these few standard compounds have constitutions which have not been determined unequivocally. The absolute method has been extremely difficult to use—actually it has found little conclusive application as yet—because of the uncommon difficulty of getting all possible isomers in order to validate the mathematical and structural predictions.

With the growth of studies on furfural and its derivatives, new tools in the form of new derivatives and reactions are rapidly making it possible to find an answer to orientation in the furan nucleus or to rules underlying the positions taken up by entering substituents. Two of these new synthetic approaches will be discussed in this preliminary report.

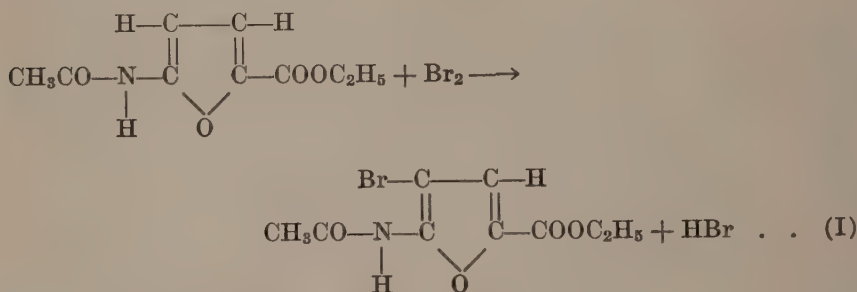
It has long been known that furfural,



<sup>1</sup>This is one of a series of studies in organic chemistry concerned with the utilization of agricultural wastes. The authors gratefully acknowledge assistance from the Industrial Science Research Fund for the defrayal of expenses incurred in this investigation.

<sup>2</sup>Gilman and Wright, *Iowa State College Jour. Science*, 4, 35 (1929); *J. Am. Chem. Soc.*, 52, 1170, 2550, 4165 (1930).

and other readily accessible mono-substitution products like furoic acid (where the substituent is in the (2) or (5) position) undergo relatively ready substitution in such a manner that the new entering group takes up position (5) (or (2)). An explanation for this type of substitution based on a 1, 4-addition, was offered recently<sup>3</sup>. At that time, attention was directed to the fact that it is astonishingly difficult to get the second substituent in the (3) or the (4) position. This can now be effected by the following series of transformations:



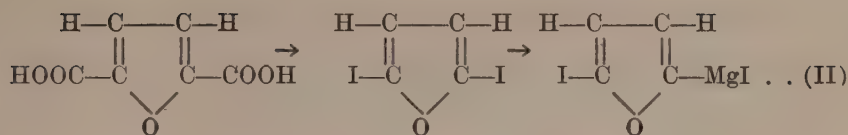
That is, the acetamino group in position (5) makes it possible, as one might have predicted, to replace a nuclear hydrogen by halogen. This reaction takes place with an ease that is refreshing and with yields that are highly satisfactory when one recalls the drastic conditions and depressing yields in related substitution reactions when the two substituents already present are of a so-called negative nature. The introduction of a nitro group into ethyl 5-acetamino-2-furoate is also readily effected. We do not know at this time the position taken up by the new substituent: it may be (3) or (4) and we are tentatively suggesting that it is (4). An answer to this question will be forthcoming directly, because it should prove possible to reduce the ethyl 3-(or 4)-nitro-5-acetamino-2-furoate to the corresponding amino compound, which after acetylation should undergo ready substitution to the 4 (or 3) position<sup>4</sup>. In this manner, the entire necessary series of substituents would become available and then the absolute method for determining orientation would enfold. The value of such transformations is not restricted to information on orientation, but would extend to the synthesis of a miscellany of nuclear substitution products and so place furan chemistry on a basis (and, it is hoped, importance) with benzene and related aromatic chemistry.

The second synthetic approach lies along a path leading from the now accessible dehydromucic acid, through the following reactions:

<sup>3</sup>Gilman and Wright, *J. Am. Chem. Soc.*, 52, 3349 (1930).

<sup>4</sup>Obviously an intermediate transformation might be used. For example, the ethyl 3- (or 4-) nitro-5-acetamino-2-furoate could be hydrolyzed to the corresponding amine; then, this nitro amine could be diazotized, and the diazo group replaced by another (or coupled or reduced) to give a miscellany of nuclear tri-substituted furan compounds.





It has now been shown that the 2, 5-di-iodofuran reacts with the magnesium-copper alloy to give 5-iodo-2-furylmagnesium iodide. By means of this Grignard reagent it will prove possible to introduce the widest possible variety of groups, inasmuch as it has been observed by some that the organo-magnesium halides are veritable touch-stones in organic chemistry because of their extraordinary facility of reaction which is reflected in a capacity to undergo reaction with practically all functional groups.

Fortunately, these two reactions will admirably supplement each other. For example, it should prove possible to synthesize a variety of halogen compounds by reaction (I), and these halogen compounds (after protection or removal of other reactive substituents) would be convertible to  $\text{RMgX}$  compounds. On the other hand, it will be possible by means of reaction (II) to prepare a series of 5-iodofurans wherein the second substituent is one introduced by means of the  $-\text{MgI}$  group. When the  $-\text{MgI}$  group in 5-iodo-2-furylmagnesium iodide is replaced by a group insensitive to  $\text{RMgX}$  compounds (like a hydrocarbon radical, dimethylamino, and other groups) then the iodine in position (5) will undergo reactions, through the  $-\text{MgI}$  compound formed from it, to give 2, 5-disubstituted furans.

## EXPERIMENTAL PART

### BROMINATION AND NITRATION OF ETHYL 5-ACETAMINOFUROATE

The ester was prepared by catalytic reduction of ethyl 5-nitro-2-furoate. This method is more convenient than that described by Marquis<sup>5</sup>, and the yields are greater. Bromine in carbon disulfide was added to the ester suspended in a solution of equal parts of carbon disulfide and chloroform. The bromo compound was obtained by removal of the solvent under reduced pressure. When crystallized from hot benzene it melted at  $112^\circ$ . The yield was 80%.

*Analysis:* Calc. for  $\text{C}_9\text{H}_{10}\text{O}_4\text{NBr}$ ; Br, 28.95%; Found 28.49%.

The nitration of ethyl 5-acetamino 2-furoate was effected by adding a suspension of the ester in acetic anhydride to the chilled nitrating mixture in the customary manner<sup>6</sup>. The product partially precipitated in the reaction mixture and was filtered off. The filtrate was washed with water and dilute sodium hydroxide solution to remove excess acid and was then coagulated with pyridine. The yield of crude product melting at  $136^\circ$  was 52.5% of theoretic. This was recrystallized from hot alcohol and was found to melt, when pure, at  $138^\circ$ .

*Analysis:* Calc. for  $\text{C}_9\text{H}_{10}\text{O}_6\text{N}_2$ ; C, 44.63%; H, 4.14%. Found: C, 44.66%; H, 4.25%.

<sup>5</sup>Marquis, *Ann. chim. phys.*, (8) 4, 196 (1904).

<sup>6</sup>Gilman and Wright, *J. Am. Chem. Soc.*, 52, 2550 (1930).

## PREPARATION OF THE MONO-GRIGNARD REAGENT FROM 2, 5-DI-iodofuran

The di-iodofuran<sup>7</sup> was prepared from dehydromucic acid<sup>8</sup>. Its reaction with re-activated magnesium-copper alloy<sup>9</sup> gave a Grignard reagent<sup>10</sup> which showed a positive color test<sup>11</sup>. Carbonation<sup>12</sup> yielded 5-iodo-2-furoic acid, melting at 192°.

*Analysis.* Calcd. for  $C_5H_3O_3I$ : I, 53.36%. Found: I, 52.89%.

Neutralization equivalent. Calcd: 238. Found: 235.

## SUMMARY

Two types of reaction have been described which will make it possible to extend markedly the preparation of nuclear substituted furan compounds, particularly those having substituents in the hitherto relatively inaccessible (3) and (4) positions. By means of these and related reactions it will be possible to determine orientation in the furan nucleus both by the absolute method and by extensions of the relative or reference method.

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<sup>7</sup>Phelps and Hale, *Am. Chem. J.*, **25**, 445 (1901).

<sup>8</sup>Yoder and Tollens, *Ber.*, **34**, 3447 (1901).

<sup>9</sup>Gilman, Peterson and Schulze, *Rec. trav. chim.* **47**, 19 (1928).

<sup>10</sup>Grignard reagents from furan types have been prepared only recently. See Gilman and Dickey, *J. Am. Chem. Soc.*, **52**, 2144 (1930) and Shepard, Winslow and Johnson *ibid.*, **52**, 2083 (1930).

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<sup>12</sup>Gilman and Parker, *ibid.*, **46**, 2816 (1924).

# THE ACTION OF CITRIC ACID AND ITS SALTS IN SUGAR SOLUTIONS<sup>1</sup>

J. H. TOULOUSE WITH J. H. BUCHANAN AND MAX LEVINE

*From the Departments of Chemistry and Bacteriology, Iowa State College<sup>2</sup>*

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## INTRODUCTION

Recognition of the fact that yeast are the principal cause of spoilage in carbonated beverages (1) led to a study of methods whereby a sterile product could be obtained. The work of Buchanan, Levine, McKelvey and Peterson (2), (3), (4), laid the foundation for a hot process which was entirely successful in producing sterile syrups in regular commercial practice. The chief objections to this process came from those manufacturers who did not have available the means to properly carry out the procedure. There was also the fact that a hot process syrup could be recontaminated if not carefully handled, and could, therefore, be subject to the same kind of spoilage as before. The present paper is a report of a method which overcomes part of these difficulties.

As a result of the study of the hot process, it was recommended that the syrup be boiled for ten minutes to insure sterility. If citric or tartaric acid was added in quantities equivalent to that desired in the final beverage, sterilization could be effected by merely bringing the syrup to a boil.

A method of sterilizing syrups by other agencies than heat presented itself through data collected in a study of carbonated beverages. It was noticed that when a bottler submitted samples of syrup for the purpose of locating the cause of a particular spoilage, the simple syrup (sugar and water alone) often contained a much larger number of living yeast than the flavored syrup (simple syrup with the addition of flavor, color and acid solution). The number of yeast in the latter often approached zero. Table 1 shows some of the data obtained.

The data tabulated in table 1 included only syrups in which the acid concentration was high. None showed a pH greater than 2.18. In addition to the above samples a large number of other syrups upon which data were also recorded included information upon the beverages made from them. In one series of these syrups, in which the simple syrups were all highly contaminated, the flavored syrups were all either sterile or the yeast were greatly reduced in numbers. It was found that beverages made from these syrups were all more acid than the acidity indicated by pH 3.2. Another group in which the simple syrups were of the same degree of contamination, but in which the flavored syrups made from them showed an equal or

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<sup>1</sup>A thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup>These studies were made possible through a Fellowship maintained by the American Bottlers of Carbonated Beverages at Iowa State College.

TABLE 1. *Yeast content of simple and flavored syrups. The flavored syrup in each case is from the simple syrup shown on the same line.*

Simple syrup			Flavored syrup		
Living yeast per cc.	Sp. gr.	pH	Living yeast per cc.	Sp gr.	pH
15,000	1.276	5.6	18	1.262	2.0
12,000	1.252	6.1	0	1.248	2.11
12,000	1.252	6.1	6	1.2414	2.15
10,000	1.287	----	0	1.1737	1.93
40,000	1.226	5.9	4	1.2081	2.18
100,000	-----	----	0	-----	1.79
25,000	1.283	----	44	1.280	2.12

greater number of yeast than their respective simple syrups, were found to contain so little acid that the beverages made from them were of pH 3.6 or greater. The beverages in the latter group were without exception in a more advanced stage of spoilage at the time tested than those of the first group.

The above points to the possibility of a "cold process" based upon definite conditions of time and of acid concentration. Since citric acid is used to acidify almost all beverages its use under carefully determined procedures can be a definite part of the process of manufacture.

These observations led to the thought that a careful study of the factors influencing the rate of death of yeasts in heavy sugar solutions with various concentrations of acid and acid salts might throw considerable light on the mechanism of sterilization of syrups. With this in view the following experiments were carried out.

The plan of investigation was:

1. To observe effect of time, concentration of sugar and strength of citric acid on the killing of yeast (both vegetative and spore forms) in heavy sugar syrups.
2. To observe the effect of various salts of citric acid upon the killing time.
3. To observe, in particular, the effect of small amounts of sodium dihydrogen citrate on the death time of yeasts in sugar-citric acid-water media. This combination of citric acid and salts might readily be encountered in syrups made from highly alkaline waters.
4. To observe changes in pH, conductivity, and inversion of sugar when citrates are added to sugar solutions acidified with citric acid.

#### HISTORICAL AND THEORETICAL

In summing up such work as has been reported having a bearing on this subject, two things should be kept in mind. The first has to do with known effects of acids, or acid salt systems upon micro-organisms in general, and upon yeast in particular. The other includes the properties of acid and acid salt systems in water alone and in sugar solutions. From



either source some light might be expected which would have a bearing upon the subject.

*A. The effect of acid and of acids and their salts on the growth or death of yeast*

Many authors could be cited with reference to a difference in the acid tolerance between yeast and bacteria. In order to establish the growth ranges of each, some reference to the limiting reaction for certain bacteria should be made.

The only case of an acid salt system compared with a growth phase of a micro-organism, was found in the work of Van Dom (5). He studied the influence of lactic acid and sodium lactate on lactic acid fermentation. Two limits were found: (a) the acidity of the medium, (b) the amount of undissociated lactic acid. His method was to add amounts of lactic acid, sodium lactate, or combinations of both, to a lactose medium, which was then inoculated with lactic acid bacteria and allowed to remain until growth had ceased. He then measured the pH and total lactate of the end products. Two factors causing death of the organism were found, each independent of the other. If enough lactic acid was present to give a pH of 4.0 growth ceased. If enough sodium lactate was present to cause the amount of undissociated lactic acid to reach 0.01 N, growth also ceased.

Grove (6) also established limits, in terms of the percentage of acid, for the growth of the "cider sickness bacillus." No acid used required more than 0.5 per cent concentration to prevent growth. Re-calculating from his figures, the pH of those concentrations of acid preventing growth we obtain the following:

Limiting concentration of acid	Percentage	pH
Sulfuric .....	0.05	2.07
Salicylic .....	0.07	2.72
Tartaric .....	0.3	3.00

These different values for the pH would indicate that some factor other than concentration of the hydrogen ion must be involved.

Kolthoff also points out that the undissociated molecule has a bearing on the growth of bacteria. He states that "growth in cultures is brought to a standstill, not by the hydrogen ion concentration, but by the undissociated acid formed by the growing bacteria."

Turner (7) reported upon a series of 14 groups of yeasts isolated from carbonated beverages. These groups varied in their growth characteristics, but growth was reported in some groups in a medium with a reaction as acid as pH 2.3, and as alkaline as 12.0. There were some organisms that could not stand an acidity of more than 3.3, and others that could not grow in a medium more alkaline than 7.2. Others showed some degree of growth over the whole range upon which experiments were carried out. The optimum pH range in most cases was between 4.3 and 5.9. Peterson (8, 9) also reports some work with acid and high heat, but no values for the reaction of the medium are given. The time required to sterilize syrup of 36° Be. density with the addition of one cubic centimeter of 7.074 N citric acid to 100 cubic centimeters of this syrup was less than two minutes. The same

syrup without acid required 28 minutes to become sterile. The temperature of both experiments was 100° C.

In work of a nature similar to that of Turner (7), Svanberg (10) finds the optimum range for bottom yeast to fall between a pH of 4 and 6, the optimum range for top yeast between a pH of 3 and 6, and for torulae between 2.5 and 6.

Somogyi (11) also noted the effect of acids on yeast fermentation in 10 per cent syrup. He decided that the hydrogen ion concentration was not the only factor, but that other characteristics should be considered. He lists swelling, surface tension and flocculating powers as factors having influence.

Clark (12) points out that inhibition of yeast growth is not a function of the  $H^+$  ion concentration alone.

That yeast can tolerate a high degree of acidity is shown by the work of Kataguri (13). Fermentation was found to take place in acetic acid solutions with a concentration as high as 0.5 N. The pH of this strength of acetic acid is 2.55 according to tables quoted in Van Nostrand's Chemical Annual (14). He found that at constant concentration of acid, the rate of fermentation is almost independent of total acetate concentration.

No work seems to have been reported by any author relative to the effect of salts on acids in strengths capable of killing yeast or inhibiting their growth. Except for the work on lactose fermentation already referred to, no experimental work of this nature was found in the field of bacteriology.

Wagner (15) found that the richer the medium was in nutrient the higher acidity the yeast could withstand.

In summarizing the foregoing it should be especially noted that most of the authors cited brought out in one way or another the fact that pH was not the sole limiting factor in the growth of yeast or bacteria. The anion and the undissociated acid have very important parts. That the anion is not always a factor of definite germicidal value, can be established from the fact that the salts of an acid in question, even in high concentrations, are not germicidal until some slight acidity is formed by the organism. In Van Dom's work on the part of the undissociated acid, it was brought out that at different salt concentrations, giving different amounts of the (lactic) anion, the product of the  $H^+$  ion concentration and the lactate-ion was the same in all solutions after fermentation had ceased. This would indicate that both reaction and concentration of undissociated acid had a part to play and were mutually inter-dependent. This interpretation should modify his statement that the limit first to be reached, whether pH or concentration of undissociated acid, was the controlling factor.

An analogy to the action of acid in killing yeast is found in the work of Buchanan, Levine and associates (16, 17, 18, 19) on the killing power of solutions of sodium hydroxide and its salts. It was reported that although sodium hydroxide was found to have very high germicidal powers, these properties were greatly increased when sodium carbonate, sodium phosphate or sodium chloride were added. These salts were in themselves almost inert in so far as their killing power for bacteria was concerned. In a series of experiments in which sodium hydroxide was the first member, and in which sodium hydroxide with the addition of increasing amounts

of different salts made up the remaining members, the phenomenon of increasing germicidal value with no determinable change in hydrogen ion concentration was observed. When the increase in the undissociated sodium hydroxide was calculated from the mass law, there seemed to be a constant relation between the undissociated portion so calculated and the time necessary to kill 99.9 per cent of the bacteria in the initial inoculum. In a series in which the same pH was established, there was a wide range in the killing times.

*B. The effect of added salts on the properties of acids with and without the addition of sugar*

The effect of added salts on the properties of an acid has been studied for some time. There has been a great deal of discussion as to the value of potential and conductimetric measurements as indices of activity, of hydrogen ion concentration and of dissociation. The term activity has been applied to the values obtained by potential measurements, instead of considering these as a measure of hydrogen ion concentration. The evidence presented here is given to advance possible reasons for change in yeast-killing powers of acids with the addition of salts.

In 1899 Arrhenius added to his dissociation theory the concept that salts increase the dissociation constants of weak acids. McBain and Coleman (20) recalculated his results and found this effect to be lacking and the original concept to be in error.

Later McBain and Kam (21) offered further evidence toward the solution of this question. In the case of acetic acid the presence of NaCl gave higher potential readings than for acetic acid alone. This had been interpreted to mean that a greater dissociation had taken place. In the light of vapor pressure determinations, based upon the amount of acetic acid with and without the addition of salt, these authors find that there is an increase in the amount of undissociated acetic acid, rather than a decrease. They find an increase in the undissociated acetic acid activity almost equal in value to the increase in  $H^+$  ion concentration as measured by the hydrogen electrode potential measurements.

Brönsted (22) states that the addition of salts to a solution of weak acid decreases the activity coefficient, but increases the activity of the undissociated molecules. The net result, if the acid is weak enough, is that the total activity will be increased. In the case of stronger acids (hydrochloric and sulfuric in chrom tanning liquors), Thomas and Baldwin (23) find chlorides increase the  $H^+$  ion concentration, while sulfates cause a decrease. In a system composed of acetic acid, sodium acetate, and water, Schreiner (24) found good agreement between potential and conductivity measurements.

When sucrose is added to a system of acid, salt and water, there is often shown an increase in the activity of the solution. This is shown in some of the references cited below. Some of these authorities prefer to call the phenomenon an increase in the activity of sucrose. Others explain it on the basis of the decreased solvent material present when sucrose has been substituted for solvent. Calculations are cited below to show "hydration" of sucrose with six to eight molecules of water.



Some very definite values for the hydration of the added salts are given by Whympster (25). He finds that the presence of salts increases the velocity constant of inversion of sucrose by nitric acid, but that the effect is balanced by the addition of a certain amount of water. If one mol of  $\text{HNO}_3$  and 0.5 mol cane sugar are dissolved in 1000 grams of water, the velocity constant is 465. If one mol of  $\text{AgNO}_3$  is added to this system the velocity constant becomes 531. But if one mol  $\text{AgNO}_3$  and five mols of water are added to the system the constant of velocity remains at the original value. From other determinations of this kind he finds the following "hydration values" which are simply the mols of water which when added along with the salt, produced no change in the velocity coefficient:

Average hydration values per mol of added salt

Sugar plus	Ag	$\text{NH}_4$	K	Na	Ba	Ca	Sr
HCl		10	10	13	19	22	
$\text{HNO}_3$	5	7	8	1			18

This offers an explanation of the added effect solely on a basis of concentration change. It will be noted that the above experimental work is with strong acids.

Kolthoff (26) found that sucrose increased the pH of an HCl solution, and that the hydration of the sucrose per mol could be expressed by  $8 \pm 2$  mols of water. Scatchard (27, 28) also concludes that sucrose becomes hydrated with six or seven mols of water. Other authors (29, 30, 31, 32) point to the apparent increased activity of the solution upon the addition of neutral salts as a case of displacement of solvent. Jones and Lewis (33) offered a complete explanation based upon the stoichiometric loss of water for the increased  $\text{H}^+$  ion activity in solutions containing added sucrose, as though the sucrose were a chemically inert diluting material. They question the presence of hydrated sucrose.

A distinction should be made with respect to strong and weak acids. While a definite increase in the rate of hydrolysis of sucrose has been demonstrated in the case of strong acids in the presence of their salts, the reverse is true in the case of weak acids. Salts of weak acids decrease the rate of inversion even in the presence of strong acids, because of the double decomposition taking place, ending with the formation of the weak acid and salt of the strong acid (34). Statements are made that the addition of salts with a common ion (35) increases the rate of hydrolysis, but this is found to be true only in the case of strong acids.

The references cited have been those dealing with the effect of acids with and without the addition of their salts, on sugar solutions and on micro-organisms found in these solutions. It has been shown that the germicidal value of acids are affected by salts of these acids in a very definite way. Sugar also affects the activity of an acid or an acid-salt system. There is a change in reaction, in inversion, in activity and in germicidal value when salts are added. Some analogy from other systems must be brought to bear on the effect of acids and their salts upon yeast. Bacteria, being less acid tolerant, furnish one field for comparison. The effect of alkalis and their salts furnish another, while the effect of acids and salts fur-



nish a third. Back beyond all of this, the fact that  $H^+$  ion concentration alone does not satisfactorily explain either the inversion of sugar or the killing of yeast, might indicate that the anion or the undissociated part may be equally responsible for such varying results as have been recorded.

With this in view, the experimental work which follows was begun, with the plan of recording those changes in the rate of death of the yeast, sugar inversion, reaction and conductivity, as might give some light upon the subject.

### *C. Studies on yeast obtained from carbonated beverages*

It has been pointed out briefly in the introduction that yeasts have been considered responsible for much of the spoilage in bottled carbonated beverages. A more detailed explanation should be given at this point.

Upton (36) pointed out that many of the yeasts present in bottled carbonated beverages are present in the sugars from which they were made. Owen and Kopeloff (loq. cit.) also found yeasts in sugar, and the former developed a method of treating raw sugar based upon inoculation with torulae in order to supply a small amount of carbon dioxide. De Grotte (37) stated that fruit juices used in the manufacture of carbonated beverages "contain yeasts which are common to all fruit juices" and were in a dormant or spore form.

Levine, Buchanan and McKelvey (3) reported that over 90 per cent of the spoilage encountered in examination of beverages examined by them was due to yeast. In earlier work Levine (4, 38) discusses the manufacturing procedure in beverage production with emphasis on the sanitary control of various pieces of equipment.

McKelvey (2) isolated over one hundred strains of yeast from beverages examined at the Fellowship established by the American Bottlers of Carbonated Beverages. He studied their spore production on malt extract agar, carrots and carrot agar.

Turner (7) studied fifty-one of the yeast isolated by McKelvey, and divided them into fourteen groups on the basis of their cultural characteristics. Twenty-eight of these were found to produce spores, and eighteen of the spore formers were separated into one group on the basis of cultural characteristics. Since this group represented many different sources and contained over one-third of all the yeasts studied by Turner, it might be termed the most characteristic group of yeast in carbonated beverages.

## EXPERIMENTAL

The experimental part is shown in four sections: (A) the purpose of the work proposed, (B) the source of the materials, especially the organism, (C) the technique used, and (D) the results obtained.

### A. PURPOSE

The results of observations on the sterility of simple and flavored syrups showed that badly contaminated syrups would become sterile after a short storage time if the proper amount of acid (usually in the form of citric acid) were added. It is a common procedure in bottling practice to make syrups by mixing 10 pounds of sugar with each gallon of water. The

resulting syrup has a Baume strength of about 29.5°, or nearly 55 per cent sugar. Most of the beverages based upon this syrup are made by adding one fluid ounce of 50 per cent citric acid to one gallon of simple syrup, together with the proper amount of flavor and color. Such flavored syrups may eventually become sterile as is shown by table 1.

The plan of the present work was to study the rates of death or the death time in syrup having the above composition, as well as syrups of other strengths, under the influence of varying amounts of citric acid and sodium citrate. With this information, and that concerned with the relations of density, reaction, conductivity and rate of inversion, some mechanism explaining the death of yeast might be arrived at. The experimental work will be arranged in this manner:

1. Rates of death of yeast, and death time
  - a. 24° Be. syrup
  - b. 27° Be. syrup
  - c. 30° Be. syrup
  - d. 33° Be. syrup
  - e. 36° Be. syrup
2. pH in syrups as above
3. Conductivity in syrups as above
4. Rate of inversion of sucrose in syrups as above.

## B. SOURCE OF MATERIALS

### a. *The test organism*

The yeast chosen was selected from those organisms studied by Turner (7). It was a strain that readily formed spores, and appeared to be characteristic of the group. It was chosen because of its ready spore formation and because of the well defined colonies it formed on Wort agar (Bacto). Turner has designated it as No. 11 in his discussion of yeasts from carbonated beverages. For characteristics of this and similar yeasts, Turner's thesis should be consulted.

Yeast No. 11 was isolated from a spoiled cherry soda. It easily demonstrated the spore stage, since it formed spores readily on gypsum blocks, malt and carrot agar at 22°C. It formed spores on carrots only at 28°C.

The optimum temperature for its growth appeared to be 25°C., growing better in the presence of air than in the absence, but having fair growth in either condition. Turner classified it in his group N, which comprised 18 out of 51 yeasts studied.

### b. *Inoculum*

The yeast used was passed through several platings, each time selecting one colony for suspension and replating. This procedure, together with the fact that no observable change in the organism took place during the course of the work, is presented in proof that a pure culture was used in the experiments.

For inoculation a two day malt extract broth culture was employed. At the end of this time the yeasts were well developed and there were al-

ways between six and ten million yeast per cubic centimeter. A heavy, dark brown deposit of yeast would form, and a great many yeasts in spore form could be demonstrated, even in three day broth cultures. The same is true of malt agar and carrot agar slant cultures. For this reason the inoculating solution always contained a large amount of spores, as well as vegetative forms.

### *c. The sugar, acid and salts*

The sugar used was a high grade commercial cane sugar, whose normal solution gave a reading of 99.9 when polarized in a 200 mm. tube in a saccharimeter at 20°C. The moisture content was 0.034 per cent. The syrups were made up to densities of 24, 27, 30, 33 and 36° Baume with distilled water. These are equivalent to specific gravities at 20°/20° of 1.1983, 1.2288, 1.2609, 1.2946 and 1.3303, respectively.

The citric acid and sodium citrate were both of the C. P. grade and were used from a fresh previously unopened bottle. Stock solutions were made and sterilized because of the ease of mold growth on even fairly concentrated solutions, then made up to volume after sterilization with sterile distilled water. In all solutions of acid, salt or syrup in which conductivity was to be taken, conductivity water was used.

### *d. Plating medium*

Wort agar (Bacto) was used for all plating work. The formula (stated on the label) was:

Maltose	12.75 parts	K <sub>2</sub> HPO <sub>4</sub>	1.00 parts
Malt extract	15.00 "	NHCl <sub>4</sub>	1.00 "
Dextrin	2.75 "	Peptone	.78 "
Glycerine	2.35 "	Agar	15.00 "

Of this agar preparation 50.63 grams were added to 1000 c.c. of distilled water. Since the mixture did not "set up" well, 5 grams of agar were added in addition to the above material in every 1000 cc. of medium.

## C. METHODS AND TECHNIQUE

In preparing syrups, the proper amount of sugar and water were mixed, with allowance for the salt and acid solution to be added after sterilizing. Sterilizing losses were found to be almost nil, because of heavy plugging, the short time of sterilizing, and the lowering of vapor pressure in heavy syrups. After sterilizing the sugar solutions, the acid and salt solutions were added and well mixed with the syrup. Then one cc. of a wort culture of yeast was added to each sugar solution, which now totaled 125 cc. in volume.

Syrups after inoculation were stored at 25°C. Initial counts were taken and the count by plating methods was repeated at definite intervals. At these times, the flask was well shaken and one cc. portions removed for dilution purposes. The time intervals ranged from two hours to three or four days on various solutions used.

Plates were counted at the end of 72 hours. Colonies were removed from plates representing platings at the beginning of certain runs, and again at the end. Examination of these colonies verified the maintenance of a pure culture.

Determinations of hydrogen ion concentrations were made by both a hydrogen train and the quinhydrone electrode. Both methods were used with the same normal calomel cell. The instruments used were a Leeds and Northrup student potentiometer and a pointer type galvanometer. A standard Weston cell whose potential had been checked against a certified standard was used frequently as a reference. A determination against a standard solution of potassium acid phthalate showed an error of less than 0.02 pH.

Conductivity was measured by means of a Leeds and Northrup type K bridge and a microphone hummer.

#### D. RESULTS

The first series of runs were made using 24°, 30° and 36° Baumé syrup. The concentrations of acid were 0.02, 0.04 and 0.08 M. This gave a range of three acid concentrations for each of three syrup densities.

In bottling practice the amount of added acid is expressed in ounces of citric acid per gallon of syrup. One ounce of a 50 per cent solution of citric acid is practically 0.02 molar when diluted with one gallon of syrup. Hence, 0.02 M, 0.04 M and 0.08 M can be translated directly to the commercial terms, one, two and four ounces of 50 per cent acid per gallon of syrup, respectively.

The data in table 2 give the effect of different concentrations of citric acid on the rate of death of yeast in syrups of various densities. Each solution number represents the average of two to four check determinations.

TABLE 2. 24° *Be.* syrup with 0.02, 0.04 and 0.08 Molar citric acid

Solution number	1	2	3
Molarity of acid	0.02 M	0.04 M	0.08 M
Period of storage at 25°C	Surviving yeast per cubic centimeter		
0 hours	188,000	188,000	188,000
12 hours	53,000	36,200	17,600
21 hours	24,800	12,100	5,260
32 hours	11,800	3,380	1,700
45 hours	4,860	1,200	420
	Percentage survivors		
12 hours	28.2	19.25	9.35
21 hours	13.2	6.43	2.80
32 hours	6.26	1.80	.90
45 hours	2.58	.64	.22
Time in hours to reach 99% reduction	58	41	30



TABLE 3. 30° *Be. syrup with 0.02, 0.04 and 0.08 Molar citric acid*

Solution number	13	14	15
Molarity of citric acid	0.02	0.04	0.08
Period of storage at 25°C.	Surviving yeast per cubic centimeter		
0 hours	62,000	62,000	62,000
3 hours	34,000	21,550	18,300
6 hours	14,960	9,200	7,400
9 hours	9,120	3,600	3,160
12 hours	4,640	1,260	466
15 hours	2,100	486	10
	Percentage survivors		
3 hours	51.6	34.8	29.5
6 hours	24.1	14.96	11.92
9 hours	14.7	5.81	5.10
12 hours	7.48	2.0	.752
15 hours	3.39	.784	.161
Time in hours to reach 99% reduction	20	14.5	11

TABLE 4. 36° *Be. syrup with 0.02, 0.04 and 0.08 Molar citric acid*

Solution number	25	26	27
Molarity of acid	0.02	0.04	0.08
Period of storage at 25° C.	Surviving yeast per cc.		
0 hours	68,000	68,000	68,000
4 hours	-----	24,000	16,240
8 hours	4,000	5,100	2,100
12 hours	-----	1,200	300
16 hours	300	100	20
24 hours	30	20	-----
34 hours	5	-----	-----
	Percentage survivors		
4 hours	-----	35.3	23.8
8 hours	7.06	7.5	3.1
12 hours	-----	1.76	.44
16 hours	.44	.15	.029
24 hours	.04	.029	-----
34 hours	.007	-----	-----
Time in hours to reach 99% reduction	14	11.5	10.0

In examining the results shown in tables 2, 3 and 4, it will be seen that the number of yeast dropped to 1 per cent of their original value in various lengths of time, but in direct relation to the amount of acid present. The 24° *Be. syrup* containing 0.02 M acid reached this value in about

58 hours, the 0.04 M solution in 41 hours, and the 0.08 M concentration in 30 hours. For the 30° Be. syrup the killing times are 20 hours, 14.5 hours and 11 hours, for the 0.02 M, 0.04 M and the 0.08 M, respectively. In 36° Be. syrup, the death times were 14, 11.5 and 10.0 in the same order.

TABLE 4a. *Relation of killing time for 99 per cent of initial yeast to density of syrup, citric acid content and pH*

Syrup degrees Baume	Acid concentration molar	Killing time hours	pH
24°	0.02	58	2.37
	0.04	41	2.20
	0.08	30	2.02
30°	0.02	20.0	2.25
	0.04	14.5	2.05
	0.08	11.0	1.88
36°	0.02	14.0	2.05
	0.04	11.5	1.86
	0.08	10.0	1.67

It would appear from examination of death times that doubling the concentration of acid decreased the killing time by about one-fourth. If the time to kill 99 per cent of the yeast in the 0.02 M acid solution is taken as one, the following relations appear:

Syrup concentration	0.02 M acid	0.04 M acid	0.08 M acid
24° Be.	1.00	.707	.517
30° Be.	1.00	.725	.550
36° Be.	1.00	.822	.715

The values for 36° Be. syrups appear to be out of line. When it is considered that the viscosity and other characteristics, some of which are shown later, of the syrups change rapidly above 30° Be., this change is partly explained.

When the data are rearranged according to increasing syrup concentration rather than increasing acid strength, the same order of change in killing time is demonstrated. For an acid concentration of 0.02 M the death times are 58 hours, 20 hours and 14 hours in syrups of 24°, 30° and 36° Be., respectively. For 0.04 M acid the time limits are 41, 14.5 and 11.5 hours, and for 0.08 M they are 30, 11 and 10 hours in the same syrup order.

The relation between syrup concentrations and rate of death of yeast is shown graphically in figure 1. It will be noted that there was a marked difference between the curves for the light syrup and the two other syrup concentrations. There was a definite decrease in killing time with increased syrup density. This change in killing time was many times greater in the range from 24° to 30° Be. than in the range from 30° to 36° Be. Since more acid must be added to a heavy syrup in order to have the same acid-

sugar ratio in the beverage, the above work indicates that heavy syrups will be better to use if a cold process of sterilization is to be attempted.

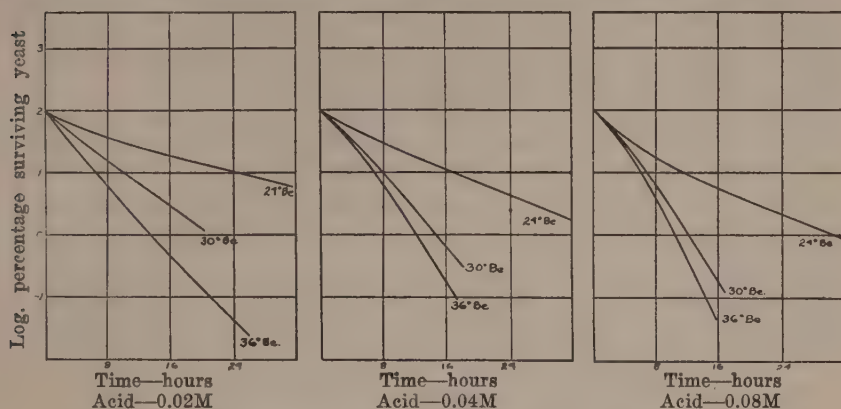


FIG. 1. Effect of syrup concentration on rate of death of yeast.

In order to compare the death time of the yeast with the acidity of the solution, determinations of the pH of different sugar solutions were made. For this purpose a highly purified sugar was used in order that the salt effect could be eliminated as far as possible. Table 5 shows the results obtained.

TABLE 5. *pH of citric acid solutions at 25°C*

Sugar degrees	pH at citric acid concentrations of			
Baume	0.08M	0.04M	0.02M	0.01M
0.0	2.10	2.27	2.44	2.62
12	2.07	2.25	2.41	2.59
24	2.02	2.20	2.37	2.54
30	1.88	2.05	2.25	2.42
36	1.67	1.86	2.05	2.23

If pH were the only factor in the death of the organisms, the time to kill 99 per cent of the organisms should be the same for the following concentrations:

1. 30° Be. syrup (0.08M acid) and 36° Be. syrup (0.04M acid)
2. 30° Be. syrup (0.04M acid) and 36° Be. syrup (0.02M acid)
3. 24° Be. syrup (0.08M acid) and 30° Be. syrup (0.04M acid)
4. 24° Be. syrup (0.04M acid) and 30° Be. syrup (0.02M acid)

The actual killing times found for the syrups listed above in the same order are:

1. 11 hours and 11.5 hours, respectively
2. 14.5 hours and 14.0 hours, respectively
3. 30 hours and 14.5 hours, respectively
4. 41 hours and 20 hours, respectively.

At first those marked 1 and 2 seem to bear out the statements made that killing power is directly related to pH. However, there appears to be a definite limiting time, lower than which it is not possible to go at the temperature and acid range used. This time is about 10 hours. Therefore, an apparent agreement might be secured simply because the values for killing time closely approached the limiting death time.

This is not true in the case of 3 and 4. In these the time in hours ranges from 2 to 4 times the limiting time seemingly established. At the same time the hours to kill 99 per cent of the organisms in 24° Baumé syrup

TABLE 6. 24° *Be. syrup with citric acid and sodium citrate equivalent to NaH<sub>2</sub> citrate*

Solution number	4	5	6
Molarity of acid	0.02	0.04	0.08
Molarity of Na <sub>2</sub> Citrate	0.01	0.02	0.04
Storage period at 25°C.	Surviving yeast per cc.		
0 days	188,000	188,000	188,000
3 days	642,000	492,000	481,000
12 days	2,040,000	1,600,000	632,000
18 days	5,260,000	2,420,000	2,462,000

TABLE 7. 24° *Be. syrup with citric acid and sodium citrate in equivalent molar quantities*

Solution number	7	8	9
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.02	0.04	0.08
Storage period at 25°C.	Surviving yeast per cc.		
0 days	188,000	188,000	188,000
3 days	492,000	378,000	326,000
12 days	714,000	442,000	432,000
18 days	1,242,000	892,000	704,000

TABLE 8. 24° *Be. syrup with citric acid and sodium citrate forming disodium hydrogen citrate*

Solution number	10	11	12
Molarity of acid	0.02	0.04	0.08
Molarity of Na <sub>2</sub> Citrate	0.04	0.08	0.16
Storage period at 25°C.	Surviving yeast per cc.		
0 days	188,000	188,000	188,000
3 days	222,000	123,000	78,500
12 days	148,000	32,000	4,750
18 days	123,000	12,000	520



are double those to kill a like percentage of the organisms in 30° Baumé syrup. The pH values, though approximately equal, are slightly different. In the cases of 3 and 4 the lower pH required the longer time to kill the organisms.

In order to find the effect of adding salts of citric acid to these tests, a number of runs were made on each of the sugar and acid concentrations already treated with acid only. The tabular results are found in tables 6 to 14.

TABLE 9. 30° *Be. syrup with citric acid and sodium citrate forming sodium dihydrogen citrate*

Solution number	16	17	18
Molarity of acid	0.02	0.04	0.08
Molarity of Na <sub>2</sub> Citrate	0.01	0.02	0.04
Storage period at 25°C.	Surviving yeast per cc.		
0 days	62,000	62,000	62,000
3 days	512,000	320,000	383,000
12 days	816,000	401,000	656,000
18 days	942,000	796,000	582,000

TABLE 10. 30° *Be. syrup with citric acid and sodium citrate in equivalent molar quantities*

Solution number	19	20	21
Molarity of acid	0.02	0.04	0.08
Molarity of Na <sub>2</sub> Citrate	0.02	0.04	0.08
Storage period at 25°C.	Surviving yeast per cc.		
0 days	62,000	62,000	62,000
3 days	274,000	263,000	288,000
12 days	188,000	179,000	156,000
18 days	147,000	164,000	119,000

TABLE 11. 30° *Be. syrup with citric acid and sodium citrate forming disodium acid citrate*

Solution number	22	23	24
Molarity of acid	0.02	0.04	0.08
Molarity of Na <sub>2</sub> Citrate	0.04	0.08	0.16
Storage period at 25°C.	Surviving yeast per cc.		
0 days	62,000	62,000	62,000
3 days	202,000	123,000	78,500
9 days	70,000	16,240	6,100
12 days	31,000	7,200	1,200

TABLE 12. 36° *Be. syrup with citric acid and sodium citrate forming disodium acid citrate*

Solution number	28	29	30
Molarity of acid	0.02	0.04	0.08
Molarity of Na <sub>2</sub> Citrate	0.01	0.02	0.08
Storage period at 25°C.	Surviving yeast per cc.		
0 hours	68,000	68,000	68,000
4 hours	-----	-----	19,600
8 hours	12,300	8,200	2,500
12 hours	-----	-----	640
16 hours	550	200	-----
24 hours	80	20	22
34 hours	25	-----	-----

TABLE 13. 36° *Be. syrup with citric acid and sodium citrate in equivalent molar quantities*

Solution number	31	32	33
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.02	0.04	0.08
Storage period at 25°C.	Surviving yeast per cc.		
0 hours	68,000	68,000	68,000
8 hours	12,400	8,000	2,800
16 hours	-----	-----	200
24 hours	70	120	60
34 hours	25	-----	-----

TABLE 14. 36° *Be. syrup with citric acid and sodium citrate equivalent to disodium acid citrate*

Solution number	34	35	36
Molarity of acid	0.02	0.04	0.08
Molarity of Na <sub>2</sub> Citrate	0.04	0.08	0.16
Storage period at 25°C.	Surviving yeast per cc.		
0 hours	68,000	68,000	68,000
8 hours	5,500	7,600	2,600
16 hours	-----	542	168
24 hours	52	60	12
34 hours	12	10	-----

TABLE 14a. *Control studies*  
(A) 24° Be. syrup

Period of storage at 25°C.	Surviving yeast per cubic centimeter		
0 days	188,000	188,000	188,000
3 days	540,000	624,000	640,000
8 days	1,620,000	2,020,000	2,400,000
12 days	3,500,000	3,800,000	4,300,000
18 days	4,880,000	4,550,000	4,110,000

(B) 30° Be. syrup

Period of storage at 25°C.	Surviving yeast per cubic centimeter	
0 days	210,000	232,000
2 days	1,625,000	2,162,000
4 days	3,560,000	3,235,000
6 days	3,545,000	3,640,000
10 days	5,930,000	-----
16 days	-----	4,876,000
21 days	4,080,000	4,924,000

(C) 36° Be. syrup

Period of storage at 25°C.	Surviving yeast per cubic centimeter	
0 hours	68,000	68,000
8 hours	14,000	22,000
16 hours	2,100	-----
24 hours	140	400
32 hours	62	110

TABLE 15. 30° Be. syrup with citric acid of various strengths

Solution number	37	38	39	40
Molarity of acid	0.01	0.02	0.04	0.08
Storage period at 25°C	Surviving yeast per cc.			
0 hours	63,000	63,000	63,000	63,000
8 hours	22,300	10,300	8,000	3,200
16 hours	-----	3,600	1,600	300
24 hours	8,640	1,490	520	35
34 hours	1,680	684	137	0
45 hours	317	30	0	

TABLE 16. 30° Be. syrup with citric acid and sodium citrate in the molecular ratio 4:1.  
On this basis there are 2 mols of citric acid to 3 mols of  $\text{NaH}_2\text{citrate}$

Solution number	41	42	43	44
Molarity of acid	0.01	0.02	0.04	0.08
Molarity of $\text{Na}_3\text{Citrate}$	0.00250	0.0050	0.01	0.02
Storage period at 25°C.	Surviving yeast per cc.			
0 days	63,000	210,000	210,000	210,000
2 days	-----	389,000	366,000	148,000
4 days	1,760,000	694,000	492,000	93,000
10 days	2,160,000	757,000	619,000	49,400
16 days	2,820,000	850,000	520,000	33,800
21 days	-----	800,000	480,000	24,200

TABLE 17. 30° Be syrup with citric acid and sodium citrate equivalent to sodium dihydrogen citrate

Solution number	45	46	47	48
Molarity of acid	0.01	0.02	0.04	0.08
Molarity of $\text{Na}_3\text{Citrate}$	0.005	0.01	0.02	0.04
Storage period at 25°C	Surviving yeast per cc.			
0 days	186,000	186,000	186,000	186,000
4 days	2,540,000	1,125,000	1,690,000	1,176,000
10 days	3,507,000	2,440,000	4,740,000	3,980,000
16 days	2,600,000	3,195,000	2,140,000	1,720,000
21 days	3,660,000	2,680,000	1,480,000	480,000

TABLE 18. 30° Be. syrup with citric acid and sodium citrate in equal molar quantities

Solution number	49	50	51	52
Molarity of acid	0.01	0.02	0.04	0.08
Molarity of $\text{Na}_3\text{Citrate}$	0.01	0.02	0.04	0.08
Storage period at 25°C	Surviving yeast per cc.			
0 days	210,000	210,000	186,000	186,000
4 days	1,342,000	1,190,000	406,000	250,000
10 days	3,045,000	3,695,000	580,000	192,000
16 days	1,940,000	1,720,000	726,000	48,000
21 days	1,690,000	1,040,000	682,000	23,480



TABLE 19. 30° *Be. syrup with citric acid and sodium citrate equivalent to disodium acid citrate*

Solution number	53	53	54	56
Molarity of acid	0.01	0.02	0.04	0.08
Molarity of Na <sub>3</sub> Citrate	0.02	0.04	0.08	0.16
Storage period at 25°C.	Surviving yeast per cc.			
0 days	186,000	186,000	186,000	186,000
1 day	396,000	251,000	-----	96,600
2 days	2,760,000	1,742,000	191,000	55,000
6 days	5,115,000	5,310,000	246,000	23,200
10 days	6,060,000	5,370,000	272,000	6,900
16 days	4,070,000	2,960,000	38,200	2,246
21 days	1,180,000	2,900,000	26,000	492

TABLE 20. 30° *Be. syrup with citric acid and sodium citrate in the molar ratio 1:3. On this basis there are 3 mols of Na<sub>2</sub>H citrate to one mol of Na<sub>3</sub> citrate*

Solution number	57	58	59	60
Molarity of acid	0.01	0.02	0.04	0.08
Molarity of salt	0.03	0.06	0.12	0.24
Storage period at 25°C.	Surviving yeast per cc			
1 day	417,000	-----	-----	-----
2 days	620,000	-----	-----	39,600
4 days	839,000	45,000	51,300	-----
6 days	1,600,000	32,600	14,500	7,750
10 days	-----	17,200	3,120	1,700
16 days	2,560,000	4,964	400	129
21 days	3,200,000	453	22	0

TABLE 21. 30° *Be. syrup with sodium citrate only*

Solution number	61	62	63	64	65	66
Molarity of Na <sub>3</sub> Citrate	0.0025	0.010	0.02	0.04	0.08	0.16
Period of storage at 25°C.	Surviving yeast per cubic centimeter					
0 days	226,000	226,000	226,000	226,000	226,000	226,000
4 days	2,230,000	1,510,000	1,216,000	410,000	58,000	34,500
10 days	4,160,000	3,400,000	3,980,000	746,000	14,400	5,860
16 days	4,300,000	4,446,000	2,860,000	1,500,000	3,641	533
21 days	4,660,000	4,082,000	2,800,000	1,220,000	260	12

TABLE 22. 30° *Be. syrup* with no added acid or salt

Solution number	67	68
Storage period at 25°C.		
0 days	210,000	232,000
2 days	1,625,000	2,162,000
4 days	3,560,000	3,235,000
6 days	3,545,000	3,640,000
10 days	5,930,000	-----
16 days	-----	4,876,000
21 days	4,080,000	4,924,000

In every case one factor stands out. The addition of a small amount of sodium citrate decreases the killing time. In the whole series shown  $\text{Na}_2\text{H}$  citrate was more toxic than  $\text{NaH}_2$  citrate. The effects of these salts are shown in figures 2, 3 and 4, for 24°, 30° and 36° *Be. salts*, respectively.

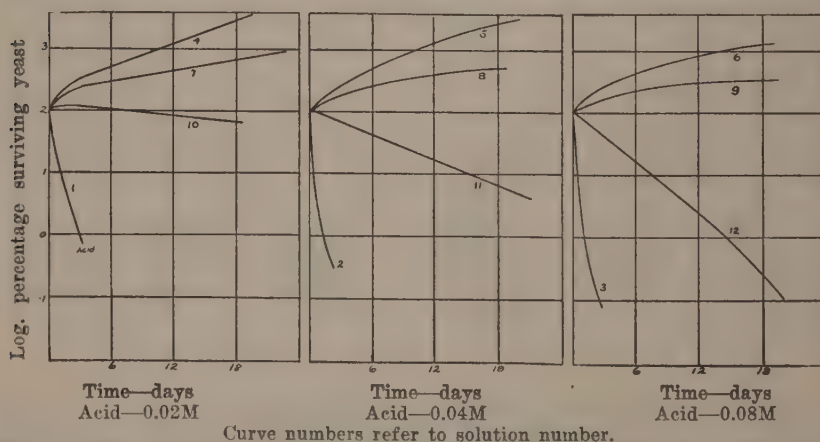


FIG. 2. Effect of sodium citrate and acid on rate of death of yeast in 24° Bé. syrup.

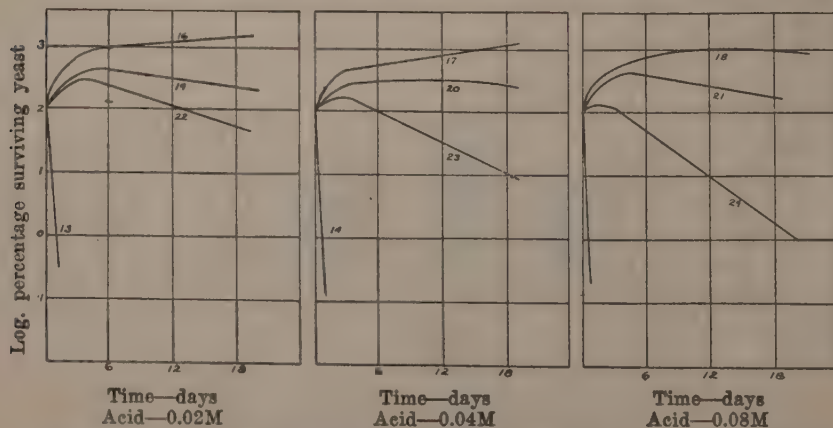


FIG. 3. Effect of syrup of 30° Bé. sodium citrate and acid on rate of death of yeast.

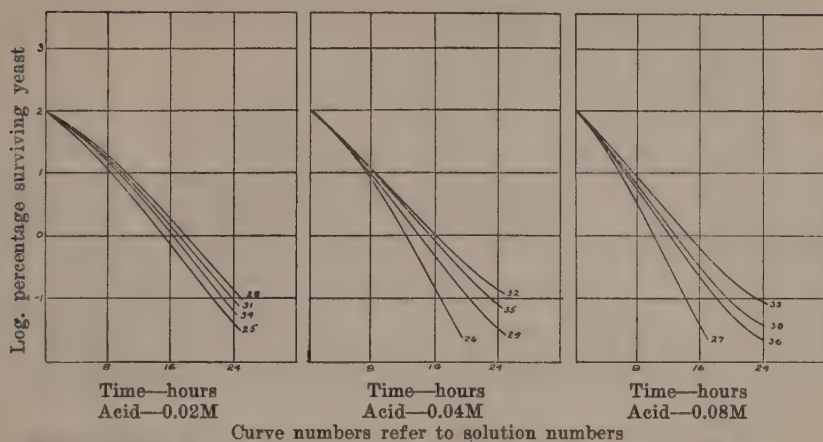


FIG. 4. Effect of sodium citrate and acid on rate of death of yeast in 36° Bx syrup.

The first table in each series of sugar densities used (tables 6, 9 and 12) show the effect of adding two parts of citric acid and one part sodium citrate. This combination will produce three parts of  $\text{NaH}_2$  citrate.

$\text{NaH}_2$  citrate proved to be almost without effect. There was no death time found and the rate of increase was almost the same as that in a culture at optimum acidity. This is not strange because the pH of the solutions ranged from 4.13 to 4.51, which is the optimum range. We can, therefore, conclude that  $\text{NaH}_2$  citrate (or the  $\text{H}_2$  citrate ion) is non-toxic.

The same observations hold in the case of the 30° Bx syrup. In the case of 36° Bx solutions there is a definite killing effect noted and this is not far from that of acid alone. This effect, however, has been found to take place in syrups of this density without the addition of any acid or acid salts.

In another series (tables 8, 11 and 14) the acid-salt ratio gave the salt  $\text{Na}_2\text{H}$  citrate. This salt showed actual toxic effect much greater than  $\text{NaH}_2$  citrate. In every case in the 24° and 30° Bx syrups, there was a definite reduction of the number of micro-organisms, and the solution should be expected to become sterile eventually. In the case of the 30° Bx syrup the killing effect was about the same as the  $\text{NaH}_2$  citrate and the citric acid alone. The  $\text{Na}_2\text{H}$  citrate (or the  $\text{H}$  citrate-ion) was more toxic than  $\text{NaH}_2$  citrate, but much less toxic than citric acid.

The third series of tables (tables 7, 10 and 13) show the effect of equal amounts of the  $\text{NaH}_2$  citrate and  $\text{Na}_2\text{H}$  citrate salts. The killing effect of the combined salts lies between the values of the two salts used separately.

The work given above was further checked by the use of 30° Bx syrup with citric acid and combinations of citric acid and its salts. It was desired to cover a more extended range and, at the same time, determine whether citric acid in the presence of its salt would have an increased germicidal effect. These results are shown in tables 15 to 22, inclusive.

Table 15 shows the acid range. Here again there was an increase in killing power of the solution with increase in acid content. The time to kill 99 per cent of the yeast ranged as follows:

13 hours for 0.08 M citric acid  
23 hours for 0.04 M citric acid  
34.5 hours for 0.02 M citric acid  
48 hours for 0.01 M citric acid

When one part of the  $\text{Na}_3$  citrate is added to four parts of citric acid, the resulting mixture has two parts citric acid and three parts  $\text{NaH}_2$  citrate. This solution, with a reserve of citric acid, should show a killing effect if there is any analogy between the killing power of citric acid with its salts and that of sodium hydroxide with its salts as found by Buchanan and Levine (loq. cit.). The reverse is true. The only one to show a decrease in the number of organisms was the one containing the highest amount of citric acid. The decrease was only 90 per cent at the end of 18 days, and it was at no time rapid. All other solutions showed increases. The pH range, however, was from 3.04 to 3.44, which was not very far from that of the citric acid alone.

Table 17, which is a repetition of earlier work over a longer time, shows the rate of death with  $\text{NaH}_2$  citrate as the acidifying medium. Here it is shown that all concentrations used allowed growth. The growth eventually began to decrease as shown in solutions 47 and 48, but this decrease came late in the period. There was no pronounced toxicity.

Table 18 shows similar action except that there is the slow killing effect of the mixtures of equal parts of the two acid salts formed by equal parts of the acid and the tri-sodium salt.

Table 19, which tabulates the results of  $\text{Na}_2\text{H}$  citrate, shows that high concentrations, as shown in solution 56, will have a definite killing power. This power is weak as evidenced by its requiring 16 days to reach a reduction of 99 per cent of the organisms.

Mixtures of  $\text{Na}_2\text{H}$  citrate and  $\text{Na}_3$  citrate are much more toxic, as shown by table 20. This series exhibits only a slow growth in the weaker solution (57) and deaths reaching the 99 per cent reduction in the stronger solutions about the tenth day. This combination of salts seems to be the strongest yet encountered.

The effect of  $\text{Na}_3$  citrate alone is shown in table 21. This salt appears to have a toxic effect in the two highest concentrations only. In this respect it is not as effective as its mixture with  $\text{Na}_2\text{H}$  citrate just mentioned. Table 22, which shows the results of two control tests, compares quite favorably with solutions 62 and 63 of table 21. There does not seem to be any deviation from control runs in these two solutions with tri-sodium citrate alone. The same is true with many of the solutions in tables 17 and 18.

In summarizing this group, it appears that:

- a. Citric acid is toxic to yeast growth
- b.  $\text{NaH}_2$  citrate is not toxic to yeast
- d.  $\text{Na}_2\text{H}$  citrate with  $\text{Na}_3$  citrate is antagonistic to yeast
- c.  $\text{Na}_2\text{H}$  is slightly antagonistic
- e.  $\text{Na}_3$  citrate is not harmful except in large concentrations
- f.  $\text{NaH}_2$  citrate, when added to citric acid, decreases its yeast-killing powers



The next series was with 30° Be. syrup, but with  $\text{NaH}_2$  citrate added in very small quantities. Previous combinations had been made by adding

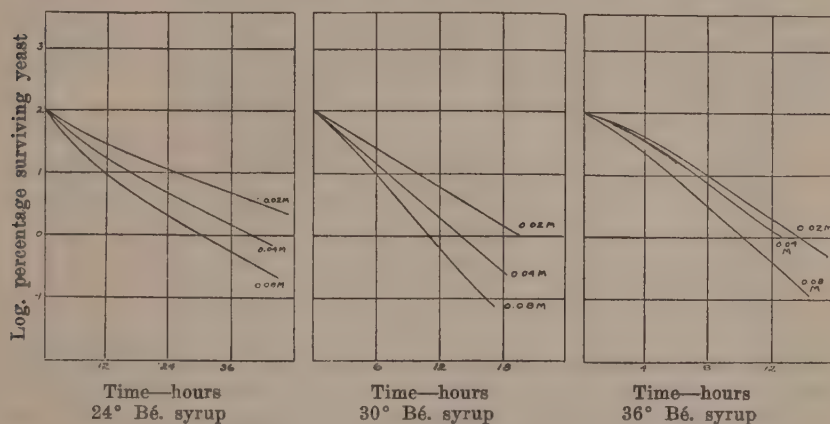


FIG. 5. Effect of acid concentration on rate of death of yeast.

TABLE 23. 30° Be. syrup with citric acid

Solution number	72	73	74
Molarity of acid	0.02	0.04	0.08
Storage period at 25°C.	Surviving yeasts per cubic centimeter		
0 hours	322,000	322,000	322,000
3 hours	318,000	309,000	295,000
6 hours	221,000	153,000	78,000
9 hours	86,700	17,640	4,300
12 hours	14,280	1,950	832
15 hours	2,420	241	160
18 hours	630	60	0

TABLE 24. 30° Be. syrup with citric acid and sodium dihydrogen citrate in ratio 5:1

Solution number	78	79	80
Molarity of acid	0.02	0.04	0.08
Molarity of $\text{Na}_2\text{H}$ Citrate	0.004	0.008	0.016
Storage period at 25°C.	Surviving yeast per cc.		
0 hours	306,000	306,000	306,000
3 hours	299,700	271,800	253,000
6 hours	196,800	116,000	72,400
9 hours	68,800	20,800	3,890
12 hours	21,200	2,508	853
15 hours	4,880	295	173
18 hours	1,424	82	
21 hours	142		

TABLE 25. 30° *Be. syrup with citric acid and sodium dihydrogen citrate in ratio 5:2*

Solution number	81	82	83
Molarity of acid	0.02	0.04	0.08
Molarity of Na <sub>2</sub> H Citrate	0.008	0.016	0.032
Storage period at 25°C.	Surviving yeast per cc.		
0 hours	224,000	171,000	224,000
3 hours	219,100	160,000	209,400
6 hours	163,300	91,800	67,600
9 hours	66,600	20,900	9,840
12 hours	28,600	5,640	693
15 hours	14,840	561	160
18 hours	1,680	22	
21 hours	196		

Na<sub>3</sub> citrate to H<sub>3</sub> citrate solutions. In the series following the NaH<sub>2</sub> citrate was made first and the acid added. In this way the amount of acid was kept at a maximum since the test was to cover the range of small additions of the di-acid salt to acid.

For reference purposes the study with citric acid alone was repeated for 30° *Be. syrup*. It was found to be necessary to run all the tests upon which direct comparison was wanted from the same culture and under identical conditions.

This series with acid alone was quite similar to the preceding series. The data are shown in table 23. In table 24 is shown the result when one part of NaH<sub>2</sub> citrate was added to five parts of the acid. The death rates are noticeably slowed down. The time to kill 99 per cent of the organisms on solutions of 0.02 M, 0.04 M and 0.08 M acid is reduced from 15 hours to 16.5 hours, from 11 to 12 hours and from 10 to 11 hours, respectively.

A similar reduction takes place when the NaH<sub>2</sub> citrate quantity is doubled. The time to kill 99 per cent of the yeast becomes 18, 13.5 and 12 hours in the same order as given in the preceding paragraph. These results are shown in table 26 and in figure 6.

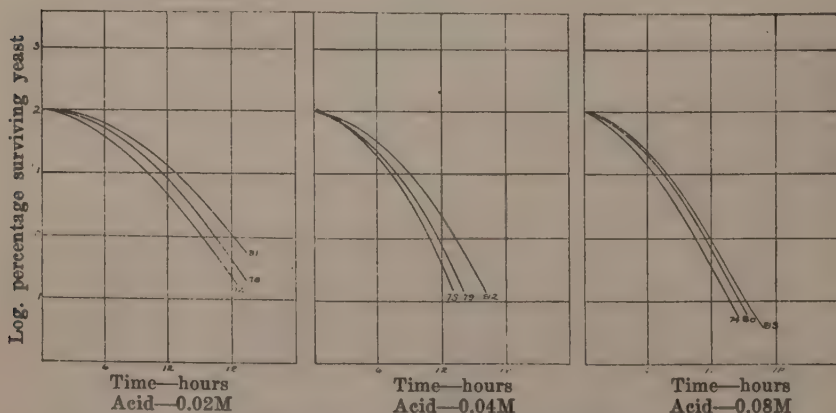


FIG. 6. Effect of NaH<sub>2</sub> citrate on rate of death in acidified 30° Bé. syrups.

TABLE 26. *Inversion and electrometric data on syrups made with citric acid and sodium di-hydrogen citrate. Inversion after 10 minutes at 100°*

Syrup Baume	Molarity of acid	Molarity of salt	Percentage inversion	Syrup pH after inversion
24	0.02	0.0	94.43	2.32
	0.02	0.04	73.5	3.22
	0.04	0.0	97.42	2.28
	0.04	0.08	88.48	3.10
27	0.02	0.0	96.09	2.42
	0.02	0.02	74.12	3.27
	0.04	0.0	97.42	2.34
	0.04	0.04	82.84	3.14
	0.08	0.0	98.41	2.20
	0.08	0.08	92.45	3.04
30	0.02	0.0	97.08	2.53
	0.02	0.04	61.0	3.28
	0.04	0.0	97.75	2.46
	0.04	0.08	73.5	3.17
	0.08	0.0	98.74	2.28
	0.08	0.16	83.5	3.08
33	0.04	0.0	97.42	2.56
	0.04	0.08	68.9	3.23
	0.08	0.0	97.75	2.35
	0.08	0.16	79.5	3.10

Table 26 is given to show the results of inversion studies. In each case the first of each pair of lines represents inversion by acid alone. The second line denotes inversion by  $\text{NaH}_2$  citrate.

It is generally accepted that the rate of inversion of sugar is directly a measure of the activity of the hydrogen ion. If this were true of sugar, the results might be applied to the study of the rate of death of yeast.

Although there is a difference between the first and second members of each pair, the second member shows good inversion always. This inversion is in good accord with the measured hydrogen ion concentration made by the quinhydrone and hydrogen electrodes. On the other hand, there was a wide difference between the killing powers of these two solutions. It would appear that hydrogen ion concentration is not the sole agent in the killing of these organisms.

Table 27 shows some relations between killing time and pH for acids in various syrups. These are compiled from previous tables and are given here to serve as a basis for figure 7, which follows. This is in support of the statement advanced earlier, that there is a limiting death time (about 10 hours) toward which all values approach. Figure 8 shows similar data for the effect of added  $\text{NaH}_2$  citrate on the rate of death of yeast in acid-sugar media.

TABLE 27. *Relation of killing time for 99 per cent of initial yeast to density of syrup, citric acid content and pH*

Syrup degrees Baume	Acid Concentration molar	Killing time hours	pH
24°	0.02	58	2.37
	0.04	41	2.20
	0.08	30	2.02
30°	0.02	20.0	2.25
	0.04	14.5	2.05
	0.08	11.0	1.88
36°	0.02	14.0	2.05
	0.04	11.5	1.86
	0.08	10.0	1.67

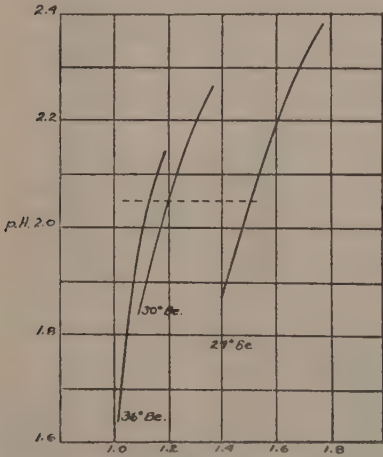


FIG. 7. The relation between pH and killing time for yeast in syrups of different densities.

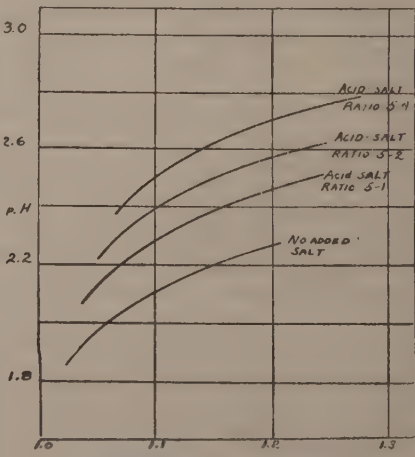


FIG. 8. Effect of salts on killing time of yeast in citric acid solutions in 30° Bé syrup

DISCUSSION

It will be seen from the foregoing figures and tables that there is a distinct change in the death rate of yeast in sucrose syrups when certain factors are varied. These factors may be sucrose concentration, acid concentration, or the amount and kind of added salt.

Figures 1 to 5, inclusive, cover a series in which three quantities of added citric acid were used with 24°, 30° and 36° Baumé syrup. These acid concentrations were 0.02, 0.04 and 0.08 molar citric acid and corresponded to the manufacturers' designations of one, two and four "ounces of citric acid per gallon" of syrup. To these acid concentrations, various amounts of tri-sodium citrate were added. The result was that in addition



to solutions containing acid alone, there was for each acid strength of each syrup density, one solution of  $\text{NaH}_2$  citrate, one of  $\text{Na}_2\text{H}$  citrate and one containing equal quantities of both these acid salts.

For each syrup concentration involving acid and acid-salt concentrations the same general facts were noted which can be observed from an inspection of the curves:

1. Acid alone is found to be toxic to yeast in all of the concentrations employed.

2.  $\text{NaH}_2$  citrate was not toxic to yeast in any of the concentrations employed. Curves for 36° Baumé syrups show deaths which can be explained as due to the high concentration of the syrup rather than to the salt alone.

3.  $\text{Na}_2\text{H}$  citrate was found to inhibit yeast growth in the lower concentrations and to cause a slow death rate at higher concentrations. That its effect is not due to pH can be shown by the fact that its range of acidity was that of the usual optimum for the yeast studied (pH 4.5 to 5.9).

Figure 1 shows the results arranged in the order of increasing syrup concentration at the same acid concentrations. It will be noted that the increase in death rate is regular with increased syrup concentrations. In passing from 24° to 30° Baumé the time is more than halved without change of acid strength. In passing from 30° to 36° Baumé the decreased death time is very slight.

Reference to figure 5 as well as to table 26 will show the relation between killing powers of different acid concentrations at the same sugar concentration. In the weaker syrup concentrations the killing time is halved by increasing the citric acid concentration from 0.02 M to 0.08 M. It is decreased one-third by doubling the acid content. With the heavier syrup strength the relationship between acid and death time does not hold. The time by which sterilization is shortened in this syrup through the addition of acid is only a small fraction of the total time. In the case of 36° Baumé syrup various factors appear to modify the process of sterilization by acid as observed in the weaker syrup. These factors are the greater osmotic pressure, greater viscosity, changes in the hydration of the sucrose present, or other changes in the system, sucrose-water, which become important in syrup densities above 30° Baumé. There seems to be a protective action on yeast as a result of the increased syrup density. Earlier work was reported in which the heavy syrups seemed to protect yeast from the effects of heat.

This protective action results in the approach of a minimum death time beyond which increase in acid or sucrose concentration becomes increasingly less effective. This will be seen in the curves in figure 7. It is there evident that a killing time of 10 hours (log. 1) will not be far from this minimum.

Hydrogen ion concentration showed a regular and almost proportionate increase in value when the same amount of acid was added to sugar solutions of increasing density. Although this is in line with the change in death rates, there is not a simple proportionate relation. Reference to figure 7 will show three different killing times for pH values between pH 1.7 and 2.4. At pH 2.05, for example, the killing times are 13.6 hours for 36° Baumé syrup, 16.4 hours for 30° Baumé syrup, and 33 hours (interpolated) for 24° Baumé syrup. Thus, it is evident that pH alone is not an

index of the killing power, for at the same pH the killing times were markedly different with different amounts of sugar.

At pH values less than 1.7 a change takes place since the line for 36° Baumé syrup approaches the curve for 30° Baumé syrup. That this result is perhaps justified and not due to experimental error may be gathered from certain physical data obtainable for this system:

1. Sucrose is believed to become hydrated with 6 to 8 mols of water, and in 36° Baumé syrup this is enough to account for practically all the water in the system.

2. The further addition of acids or salts with their demand for hydration or solution will remove more water from the system.

3. When the demands of both the sucrose-water and acid-salt-water sub-systems cannot be fully supplied by the water present, they enter into competition for the water.

4. Unless some active solvent is present, no means will be available for the acid or salt to enter through the membrane of the micro-organism. Since it is capable of maintaining the spore state under adverse conditions, the result will be that the organism may not be killed, but will remain dormant.

5. This results in a "protective action" on the part of the heavier syrups. Evidence of this is found in the very small differences in killing time for many combinations of acid and salt in 36° Baumé syrup.

The rate of inversion of sucrose had little bearing on the rate of death of yeast at the same acid or salt concentration. Inspection of table 26 will show positive inversion taking place but little less rapidly at salt concentrations which served as actual stimuli to yeast growth, than at concentrations of acid which killed yeast rapidly.

Attempts to apply such physical constants as rate of inversion of sucrose, change in pH as measured by the quinhydrone electrode, or conductivity directly to the explanation of the change in death time failed. It was not possible to obtain definite data on the un-ionized citric acid in heavy sugar systems.

The conductivity data obtained was directly opposed to that found in the absence of sugar. On the basis of replacement of water by sucrose, a concentrating effect, tending to increase the conductance would be expected. Owing probably to a high viscosity, this factor was reversed in so far as measurable conductance was concerned. In the light of uncertain corrections for viscosity or for space factors because of replacement of water by sucrose, or for change of ionization, the conductivity data taken was not found entirely suitable for application to the citric acid-sucrose water systems as an explanation for the killing of yeast. These data are shown in table 28.

Sodium citrate alone was found to be toxic to yeast. Others have reported it as antagonistic to other cell structures and harmful in the blood stream. In addition to the growth data presented, it was found that concentrated sodium citrate and  $\text{Na}_2\text{H}$  citrate solutions used as stock solutions for this work were not easily contaminated with mold, whereas citric acid solutions as high as 1.5 molar readily supported mold growth.

The addition of  $\text{NaH}_2$  citrate to citric acid, as will be seen from the above tables, resulted in a marked reduction in death rates. That  $\text{NaH}_2$

citrate, either ionized or undissociated, has no toxic effect in itself is readily evident from those curves for solutions containing this salt alone. (Tables 23 to 25.)

Tables 23, 24 and 25 give some results of adding  $\text{NaH}_2$  citrate to citric acid. There was found to be a distinct increase in killing time over that for acid alone. Whatever effect that undissociated citric acid might have had may be balanced by other factors. There was no apparent relation to the sodium hydroxide—sodium carbonate system mentioned in the historical part. Several points of difference between these two systems may be mentioned. Chief of these might be that the pH of the acid solutions was quite close (pH 2) to the optimum reaction for the yeast (pH 4.5-5.1). In the case of the alkaline solution the reaction was about a pH of 13. Thus a small change in pH might have but little effect in the latter case and more in the former.

TABLE 28. *Conductivity data for the system sucrose-citric-acid-water*

Sugar degrees Baume	Citric acid mols per liter	Cell resistance (cell constant .156)	Molar equivalent conductance	Viscosity coefficient
0	0.08	54.10	36.0	1
	0.04	73.49	52.5	
	0.02	114.16	68.25	
	0.01	166.70	93.7	
12	0.08	92.6	21.1	1.38
	0.04	141.0	27.7	
	0.02	207.0	37.7	
	0.01	302.0	51.7	
24	0.08	229.0	8.22	2.23
	0.04	380.0	10.25	
	0.02	589.0	13.34	
	0.01	830.0	18.8	
30	0.08	613.0	3.324	3.62
	0.04	1000.0	3.90	
	0.02	1581.0	4.93	
	0.01	2066.0	7.55	
36	0.08	2136.0	.912	22.4
	0.04	4114.0	.947	
	0.02	6732.0	1.158	
	0.01	8405.0	1.855	

## SUMMARY

1. For a given concentration of sugar (from 24° to 36° Be.) the rate of death of yeast increases with increasing concentration of citric acid.
2. For a given concentration of citric acid (between 0.01 and 0.08 molar) the rate of death of yeast increases with increasing concentration of sugar.
3. There is evidence of a protective action of sucrose solutions, for the increase in death rate for a given change in concentration of acid was less in the more concentrated syrups.



4. The rates of death of yeast in sucrose solutions at the same hydrogen ion concentration (in the range from pH 1.6 to pH 2.4) varied directly with the density of the syrups. The hydrogen ion concentration found when a given amount of citric acid was added to equal quantities of 24° to 30° Be. syrups decreased with increasing syrup density.
5. The addition of sodium di-hydrogen citrate to citric acid-syrup solutions decreases the death rate for yeast in sugar solutions.
6. Sodium citrate showed some toxic effects.
7. The germicidal effect of the addition of citrates to citric acid-syrup solutions or citric acid to simple syrups is less marked at the higher syrup concentrations, indicating that the sugar exerts a protective action on the yeasts.

### CONCLUSION

By means of the addition of citric acid it is possible to sterilize sugar syrup in the cold. The time varies for different syrup concentrations, being shorter with the heavier syrups. This makes possible a process of preparing syrups for bottling, canning and other industries by simply mixing and storing a solution of sugar, citric acid and water. The acid can be maintained at a concentration sufficient to meet the needs of the product to which the syrup is added.

Storage time will vary, but on the basis of the work which is here reported, and with allowance for a factor of safety, the following periods of storage are suggested for production of syrups.

TABLE 29. *Storage time in days to produce sterile syrups from solutions of sugar and citric acid at 25°C*

Concentration of syrup	Concentration of citric acid (50% solution) in ounces per gallon		
	1 oz.	2 oz.	4 oz.
	Storage time in days		
24° Be.	5-7	4-5	3-4
30° Be.	2-3	2	1½
36° Be.	1¼	1	1

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## DETERMINATION OF ORGANIC ACIDS

### IV. A METHOD FOR THE PROVISIONAL IDENTIFICATION AND QUANTITATIVE DETERMINATION OF TWO FATTY ACIDS IN A MIXTURE

C. H. WERKMAN

*From the Department of Bacteriology, Iowa State College*

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A partition method (1, 2, 3) based upon the differential distribution of the fatty acids between two immiscible solvents has been developed for the quantitative determination of each of two known acids in a mixture. The method is intended to serve with greater accuracy and less time consumption the same purposes as the Duclaux distillation method or any of its modifications. The partition method, like the Duclaux, finds its greatest application in the determination of fatty acids in fermentation mixtures.

Fermentations frequently result in the production of one or two volatile along with one or two non-volatile fatty acids. Butyric and acetic acids, propionic, acetic and lactic acids, or lactic and acetic acids illustrate combinations of acids produced by typical fermentations. Succinic acid is commonly produced in small quantities, but cannot be determined separately from lactic acid by the partition method. Formic acid may be produced, but generally in relatively small quantities. The present paper describes a method which should prove of value to the investigator who is called upon to make quantitative determinations of the volatile fatty acids present in fermentations, and who wishes at the same time to make a provisional identification of these acids.

The partition method as previously developed employs the differential distribution of the fatty acids between an ether and water. Either ethyl, isopropyl, or isoamyl ether serves satisfactorily. The ether employed will be determined by conditions of the experiment. In the method as developed, the quantitative determination can be made only if the identity of the two acids is known.

Thus, if one is working with the fermentation brought about by organisms of the genus *Propionibacterium*, two and only two volatile fatty acids are produced, propionic and acetic. In a similar manner with other fermentations, the quantitative determination of the volatile fatty acids can be quickly and accurately carried out by means of the partition method.

If the identities of the acids are not known the method will indicate the quantitative composition of the possible combinations. Reference to figure 1b<sup>1</sup> will clarify this statement. A partition constant of 18 indicates combinations of the following volatile fatty acids in the proportions given:

- (a) propionic 70 per cent 0.1 N; acetic 30 per cent 0.1 N
- (b) butyric 31 per cent 0.1 N; acetic 69 per cent 0.1 N

Formic behaves very much like acetic acid in the partition method and if present in low concentration may be disregarded. Otherwise it may easily

<sup>1</sup>Work carried out under the general program for the utilization of agricultural wastes by fermentation.

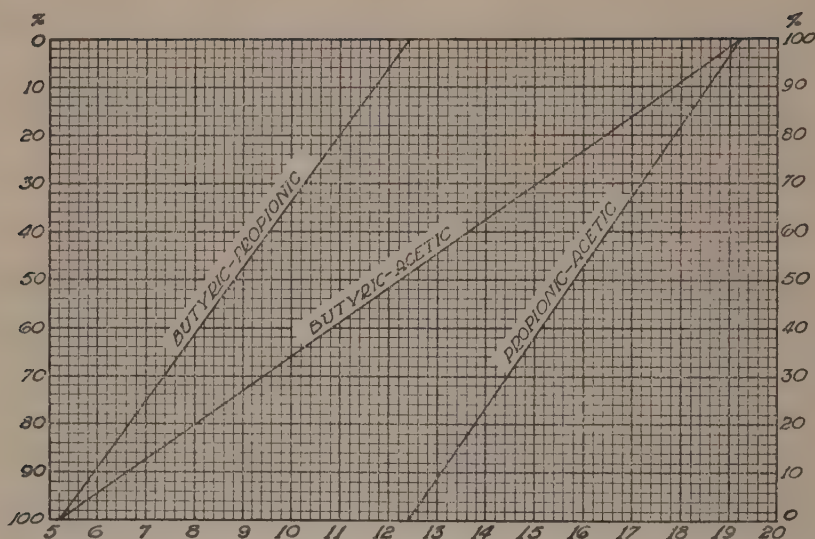


FIG. 1a. Ethyl ether partition constants

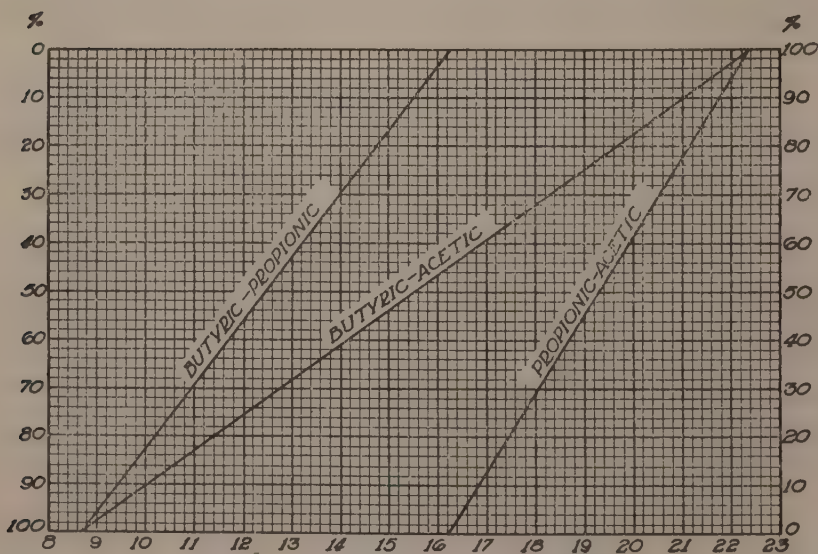


FIG. 1b. Isopropyl ether partition constants



be determined by an independent method. Formic acid generally is present only in low concentration in fermenting mixtures.

The method thus far developed does not determine which of the above combinations is the correct one. This difficulty is made apparent by the overlapping of the graphs in figure 1.

This difficulty would be obviated were it possible to find a system of immiscible solvents which would give partition constants no two of which are alike. The existence of such a system is improbable. However, if two systems whose readings of *partition constants*<sup>2</sup> when plotted against percentages of acids present, do not result in parallel graphs, are simultaneously employed, it will be possible to determine which of the two combinations of acids in the case just cited is present and also the quantitative relationships.

Such a determination may be termed a provisional identification of the acids providing only two acids are present in the mixture. At the same time the percentage of each acid present is calculated as 0.1 N acid. It is proposed to make these determinations by the use of the three systems, ethyl ether-water, isopropyl ether-water, and isoamyl ether-water. Only two of the three systems need be used; the third is treated here to provide confirmation of the results obtained by the use of the other two.

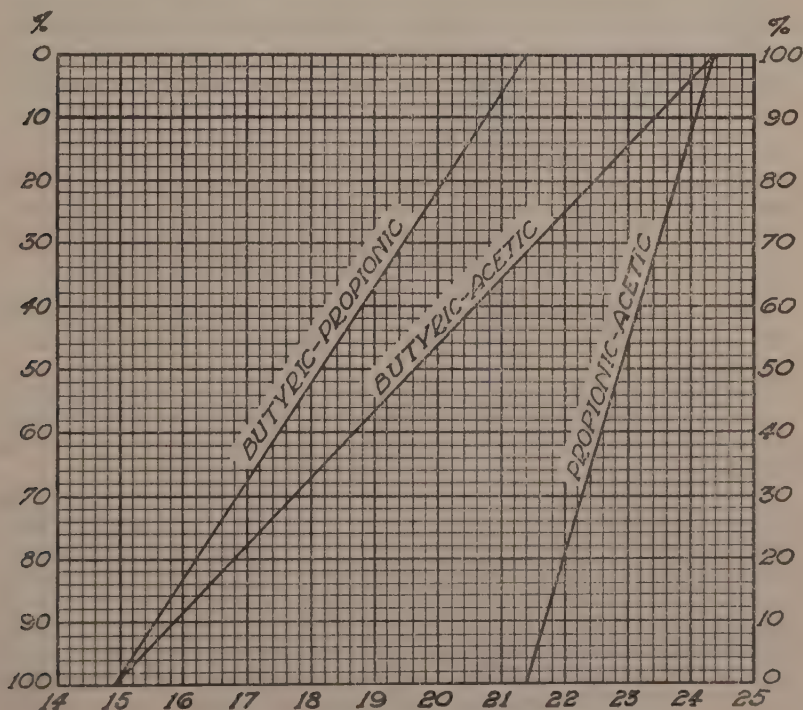


FIG. 1c. Isoamyl ether partition constants

## METHOD

To provisionally identify and quantitatively determine two fatty acids in a mixture, the unknown solution is adjusted to a convenient normality. This adjustment will be governed by the type of investigation and the strength of the solution. Generally 0.1 or 0.05 N solutions are used. Assuming adjustment to 0.1 N, 30 cc. are placed in a 100 cc. separatory funnel and 20 cc. of ethyl ether are added. The whole is shaken vigorously for one minute; three minutes are allowed for the phases to separate and then 25 cc. of the aqueous layer are withdrawn and titrated against 0.1 N alkali with phenolphthalein as an indicator. The number of cubic centimeters required to neutralize 25 cc. of the aqueous layer may be termed the *ethyl ether partition constant*.

In a similar manner the *isopropyl ether partition constant* is determined. Let it be assumed that the isopropyl partition constant is 16.8. Reference to figure 1b shows that 16.8 may be the partition constant of a mixture of 89 per cent 0.1N propionic and 11 per cent 0.1N acetic acid or 40 per cent 0.1N butyric and 60 per cent 0.1N acetic. The ethyl ether partition constant is found to be 13.7. Reference to figure 1a indicates the mixture to be 40 per cent 0.1 N butyric and 60 per cent 0.1 N acetic or 80 per cent 0.1N propionic and 20 per cent 0.1N acetic. The agreement between the readings indicates that the mixture is 40 per cent 0.1N butyric and 60 per cent 0.1N acetic. Use of the third system of isoamyl ether-water gives a partition constant of 20.7, corresponding to 40 per cent 0.1N butyric and 60 per cent 0.1N acetic or 13 per cent 0.1N butyric and 87 per cent 0.1N propionic. The reading confirms the correctness of the first conclusion.

In a similar manner other determinations are made. At times the use of two systems may fail to differentiate sharply between the two sets of possible percentages; in this case the third system must be used for the differentiation. It is suggested that the partition constants of the isopropyl and ethyl ether systems be determined first. If the results are not satisfactory and confirmation is desirable, the isoamyl partition constant is determined.

It is not necessary to confine the method to the volatile acids; mixtures of the volatile and non-volatile acids may be identified and determined quantitatively.

It is possible by means of general equations already developed (1, 2) to determine algebraically the correct combination of acids. Since the mathematical treatment would be largely a repetition, it will not be developed here.

The provisional identification and quantitative determination of organic acids by means of the partition method should prove of value in fermentation studies. It will find its greatest use in the same kinds of analytical work as that in which the Duclaux method or its modifications have been used. This applies especially to the determination of volatile acids in fermentation mixtures. The method has been used successfully to determine the acetic and propionic acids produced by microorganisms of the genus *Propionibacterium*. When used with understanding and judgment the partition method offers distinct advantages over distillation methods for the determination of fatty acids in mixtures.

<sup>2</sup>A small correction in the values of partition constants of butyric acid has been made in this paper and therefore they differ from those previously published.

## CONCLUSIONS

The partition method for the determination of fatty acids has been extended to provide a provisional identification and quantitative determination of two fatty acids in a mixture. The method should prove of value to the investigator in zymology who is required to make determinations of fatty acids in fermentation mixtures. The method will be extended to quantitatively determine three acids in a mixture.

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# THREE SPECIES OF COCCIDIA FROM THE WOODCHUCK, MARMOTA MONAX

H. B. CROUCH AND E. R. BECKER

*From the Department of Zoology, Iowa State College*

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Galli-Valerio (1923) described from the Alpine marmot (*Arctomys marmotae*) a species of *Eimeria* with egg-shaped oocysts which measured 51 by 42 $\mu$  and had a very distinct micropyle. To this coccidium he gave the name *Eimeria marmotae*.

During the summer and fall of 1930 we examined the feces and cecal contents of six woodchucks killed or captured in the vicinity of Ames, Iowa. Oocysts of coccidia much smaller than those measured by Galli-Valerio were found in all six of the animals. A study of non-sporulated, sporulating and sporulated stages has shown that three distinct species were involved. After we had prepared a manuscript on these new species, there appeared a paper by Fish (1930) in which he described *Eimeria monacis* with spherical to subspherical oocysts 16.8 to 23.2 $\mu$  in length and 15.2 to 21.1 $\mu$  in breadth; average size, 19.9 $\mu$  by 18.3 $\mu$ . No definite micropyle was observable. In our manuscript we had already designated this species *Eimeria dura* and had given the name *Eimeria monacis* to another form with ellipsoidal oocysts. Fish's paper has compelled us to revise our nomenclature and reduce the number of our new species to two.

## EIMERIA MONACIS FISH, 1930

The spherical and subspherical oocysts of this species, as determined by us, measure 14 to 20 $\mu$  in diameter. The protoplasmic mass has an irregular contour and almost fills the oocyst when it is freshly passed (Fig. 1). The oocyst as a whole appears quite clear and colorless. The wall is comparatively thick. When cultured in 2.0 per cent potassium dichromate solution at room temperature the following stages in the sporulation process are to be noted: After 16 hours, the protoplasmic mass contracts slightly and becomes smoother in outline (Fig. 2); after 26 hours, the mass appears quadrilobate with a spherical residual mass becoming differentiated in the center; after 40 hours, the oocyst holds four sporoblasts, each with a refractile mass near the surface, and a residual body about equal in size to a sporoblast; after 50 hours, the sporoblasts become elongated ovoids (Fig. 3); the process is completed within 60 or 64 hours by the formation of two sporozoites and a residual body within each sporocyst (Fig. 4). The Stieda body is prominent at the more pointed end of the sporocyst. From the fortieth hour the residual body becomes progressively smaller until finally it appears as one or several clear glary granules.

We had two animals with pure infections of this type.

## EIMERIA PERFOROIDES n. sp.

The oocysts of this species resemble somewhat those of *E. perforans* of the rabbit in shape, size and general appearance. The shape is ellipsoidal, and the size 17 to  $24\mu$  by 15 to  $20\mu$ . No micropyle is observable. The granular mass is considerably contracted from the cyst wall, even in fresh material (Fig. 5). The jelly surrounding the mass is distinctly tinted. After 20 hours development the granular mass appears somewhat square in outline. A few hours later the typical pyramidal stage appears. Separation of the pyramids may be completed within 32 hours (Fig. 6). On about the forty-eighth hour the sporoblasts become spheres with clear substance at the poles. A small residual body lying among the sporoblasts is to be noted (Fig. 7). By about the sixtieth hour the sporoblasts have become elongated ovoids. Sporulation is completed within 70 hours (Fig. 8).

This form was seen only in mixed infections along with *E. os*.

## EIMERIA OS n. sp.

This is the most distinctive of the three types of oocysts found in the woodchuck. The shape is typically ovoidal. There is an easily observable micropyle at the more tapering pole. Measurements indicate a length of 20 to  $26\mu$  and a width of 18 to  $22\mu$ . In some specimens, the endomembrane of the oocyst wall protrudes through the micropyle to the outside, forming a bulb-like swelling. The granular mass within freshly passed oocysts, or in those taken from the animal immediately after its sacrifice, is somewhat retracted from the wall, but corresponds in general to the outline of the latter (Fig. 9). The remaining area is filled with a clear pinkish jelly-like fluid. At this stage the nucleus appears among the granules as a clear sphere near the center of the protoplasmic mass.

Oocysts which have been placed in 2.0 per cent potassium dichromate solution show evidence of initiating development within 20 hours. The protoplasmic mass contracts into a compact sphere. Within 45 hours the latter becomes quadrilobate ("pyramidal stage") with clear refractile bodies at the apices of the lobes. This stage represents early sporoblastic formation. Usually only three of these lobes are to be seen in one optical plan because of their orientation within the oocyst. Between the sixtieth and seventieth hour the constrictions separating the lobes become complete, thus forming four sub-conical sporoblasts each with a refractile body at both the greater and lesser poles (Fig. 10). As in *Eimeria stiedae*, there is no oocyst residual body. During the next 10 hours each sporoblast assumes the shape of an ellipsoid (Fig. 11), measuring 9 to  $13\mu$  in length by 5 to  $8\mu$  in breadth. Some time between 90 and 105 hours two sporozoites and a residual body appear inside each sporocyst. The residual body is usually either a somewhat rounded or an irregularly biconvex mass which lies either to one side or between the sporozoites (Fig. 12). The process of sporulation is completed within 105 hours.

This species was observed both in pure and mixed infections.

TABLE 1. *Characters for separating the oocysts of the three species of Eimeria in the woodchuck, Marmota monax*

Name	Size in microns	Shape	Residual body	Sporulation time
<i>E. monacis</i>	14 to 20 (diameter)	Spherical to sub-spherical	In sporocyst and oocyst	60 to 64 hours
<i>E. perforoides</i>	17 to 24 by 15 to 20	Ellipsoidal	In sporocyst and oocyst	70 hours
<i>E. os</i>	20 to 26 by 18 to 22	Ovoidal	In sporocyst only	90 to 105 hours

It will be noted that we have shown in our figures the detailed structure of the sporozoites within the sporocysts which is ordinarily difficult, in many species impossible, to observe. Certain recent authors have not shown either the nucleus or the refractile spheres of the sporozoites in their figures. This is probably because they have faithfully represented what they actually saw. We have developed a technique for staining the oocysts which is particularly effective in bringing out the details of the mature sporocyst. The method will be published within a short time.

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## DESCRIPTION OF FIGURES

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- Fig. 1. *E. monacis*, freshly passed oocyst.
- Fig. 2. *E. monacis*, oocyst with contracted protoplasmic mass.
- Fig. 3. *E. monacis*, developing oocyst with four advanced sporoblasts and a residual body.
- Fig. 4. *E. monacis*, completely sporulated oocyst.
- Fig. 5. *E. perforoides*, freshly passed oocyst.
- Fig. 6. *E. perforoides*, oocyst with "pyramids" and residual body separating.
- Fig. 7. *E. perforoides*, oocyst with four sporoblasts and a residual body.
- Fig. 8. *E. perforoides*, completely sporulated oocyst.
- Fig. 9. *E. os.*, freshly passed oocyst.
- Fig. 10. *E. os.*, oocyst with "pyramids" separating.
- Fig. 11. *E. os.*, oocyst with ellipsoidal sporoblasts.
- Fig. 12. *E. os.*, completely sporulated oocyst.







# THE CHEMICAL ACTION OF AEROBACTER FAENI ON XYLOSE AND SUCROSE

CALVIN R. BREDEN WITH ELLIS I. FULMER<sup>1</sup>

*From the Department of Chemistry, Iowa State College*

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## INTRODUCTION

One of the fields of research now occupying the attention of various agencies is the practical commercial utilization of agricultural wastes such as the cornstalk, corncob, oat hulls and the like. One method of utilization holding promise is the production of chemicals by the fermentation of these products. The commercial production of chemicals by fermentation of agricultural products is old, but until recently was practically limited to the chemicals—ethyl alcohol and acetic acid, from the grains and fruits. Later black-strap molasses, a waste product from the cane sugar industry, has become an important source of ethyl alcohol and glycerol. One of the most significant recent developments in the field has been the large scale production of butyl alcohol and acetone by the anaerobic fermentation of corn. One plant uses thousands of bushels of corn per day in this process.

The commercial processes mentioned deal, for the most part, with the fermentation of cellulose, disaccharides such as cane sugar and maltose, or monosaccharide hexoses such as dextrose.

The agricultural waste products, such as cornstalks, corncobs and oat hulls, contain a large proportion of pentosans, which are carbohydrate materials yielding pentoses, especially xylose, upon hydrolysis. In the utilization of these materials it is necessary to deal with and develop the fermentation chemistry of the pentosans. Before proceeding directly with the pentosans it is necessary to know more about the chemism of organisms acting on a pure pentose such as xylose. Such was the purpose of this investigation. The ultimate goal would be to take a typical fermentation and make a complete study of the effect of physical and chemical environment on the kind and amount of substances produced, with a view to control of yields of valuable products.

Because of the complexity of the problem and more immediately of the unsatisfactory state of analytical methods in this type of work, it has developed that this report is preliminary in nature, covering the qualitative analysis for the products produced by *Aerobacter faeni* on xylose and

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<sup>1</sup>This communication is based on a thesis submitted by Calvin R. Breden in partial fulfillment of the requirements for the degree Doctor of Philosophy in Chemistry at Iowa State College. This research was supported by a grant from the special funds from the Industrial Science Division for investigation on the utilization of agricultural wastes. The authors wish to express their appreciation to Dr. C. H. Werkman of the Department of Bacteriology for furnishing the culture *Aerobacter faeni* and occasionally checking the purity and to Dr. R. M. Hixon of the Department of Chemistry for advice in several matters of analytical procedure.

sucrose, and a preliminary study of the relative amounts of the products produced under aerobic and anaerobic conditions.

### HISTORICAL

Comparatively few studies have been made of the fermentation of xylose, in which the dissimilation products have been adequately identified. The work that has been done is summarized in table 1. References where impure or mixed cultures were used are not included, nor are those where it was simply reported that the organism utilized xylose as a source of carbon.

TABLE 1. *Dissimilation products of xylose*

Organism	Products	Authority
Friedlander's pneumobacillus	acetic ac., ethyl alc., l-lactic ac., succinic ac.	Grimbert, 1896
Sorbose bacterium	xylonic ac.	Bertrand, 1898
Citromyces	citric ac.	Wehmer, 1913
<i>Lactobacillus pentoaceticus</i>	acetic ac., lactic ac.	Fred, Peterson and Davenport, 1919
<i>B. acetoethylicum</i>	acetone, ethyl alc.	Northrop, Ashe and Morgan, 1919
<i>B. acetoethylicum</i>	acetone, ethyl alc., formic ac.	Northrop, Ashe and Senior, 1919
<i>B. lactis aerogenes</i> <i>B. para typhoid</i> <i>B. typhosus</i>	acetic ac., butyric ac., ethyl alc., formic ac., lactic ac., succinic ac.	Fred and Peterson, 1920
<i>B. acetoethylicum</i>	acetic ac., formic ac., lactic ac., ethyl alc., acetone, CO <sub>2</sub>	Arzberger, Peterson and Fred, 1920
<i>Lactobacillus pentoaceticus</i>	acetic ac., lactic ac.	Anderson, Fred and Peterson, 1920
<i>Lactobacillus pentosus</i> <i>Lactobacillus pentoaceticus</i>	acetic ac., lactic ac.	Fred, Peterson and Anderson, 1921
<i>B. granulobacter pectinovorum</i>	acetic ac., acetone, butyl alc., butyric ac., lactic ac.	Speakman, 1923
<i>Acetobacter xylinum</i> <i>B. herbicola aurum</i> <i>B. vulgatus</i>	acetone, CO <sub>2</sub> ethyl alc.	Fred, Peterson and Anderson, 1923
<i>B. granulobacter pectinovorum</i>	acetone, butyl alc., CO <sub>2</sub>	Peterson, Fred and Schmidt, 1924
<i>Sacc. cerevisiae</i>	ethyl alc., CO <sub>2</sub> , glyceric aldehyde (?)	Abbott, 1926
<i>Asp. niger</i>	citric ac., oxalic ac.	Amelung, 1927
<i>Asp. niger</i>	citric ac., oxalic ac.	Bernhauer, 1928
<i>Propionibacterium pentosaceum</i>	acetic ac., CO <sub>2</sub> , propionic ac.	Werkman, Fulmer and Rayburn, 1929



## DESCRIPTION OF THE ORGANISM USED

The organism finally decided upon is known as *Aerobacter faeni*, Burkey, which was isolated and identified by Burkey (1928) in his studies on bacteria attacking constituents of the cornstalk. This organism, among other advantages, will grow well in a medium containing inorganic nitrogen in the form of ammonium salts. It grows rapidly on xylose, produces gas, and is relatively easy to handle.

The description of the organism as given by Burkey follows:

"Non-motile rods,  $1.0\mu$  broad and  $1.0\mu$  to  $3.0\mu$  long, conforming to the generic diagnosis. Acid and gas produced from the mono- and di-saccharides, including melezitose, from pentose sugars, raffinose, rhamnose, trehalose, salicin, aesculin, all the alcohols except erythritol, from glycogen, soluble starch, and pectin. No fermentation from amygdalin, inulin, or the pentosans. Acid and gas in litmus milk. Indol produced. Gelatin not liquefied. Isolated from hay infusion."

## EXPERIMENTAL

## A. THE MEDIUM

The medium as finally developed had the following composition:

## Medium A

Sugar	20.0 g.
NH <sub>4</sub> Cl	6.0 g.
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	1.0 g.
MgSO <sub>4</sub> ·7H <sub>2</sub> O	1.0 g.
CaCO <sub>3</sub> (pptd.)	10.0 g.

Made up to 1 liter

The pH of this medium was as made up about 7.2. The pH after sterilization was 7.0. The concentrations of the salts used were those found to give maximum utilization of xylose. The maximum amount of xylose used in this medium by *Aerobacter faeni* was about 70 g. per liter.

## B. QUALITATIVE ANALYSIS

## 1. Discussion

Since nothing was known of the nature of the products produced by this organism, other than the results of the standard tests used in classifying it, it was necessary to make a qualitative analysis of the products resulting from the fermentation. As the result of a large number of tests on the mixtures the following substances have been found: Ethyl alcohol, 2-3-butylene glycol, acetylmethyl carbinol, acetic acid, formic acid, succinic acid, l-lactic acid, CO<sub>2</sub>, H<sub>2</sub> and butyric acid. The general method used by the organic chemist in identifying the compounds in a mixture is to separate them by various physical and chemical procedures, to purify them and then to identify them by elementary analysis, determination of physical constants and preparation of derivatives. In practice this method is somewhat modified in identifying the products formed in fermentation.

Since the solutions are dilute instead of concentrated, some of the products are present only in small amounts. In this type of work sensitive specific tests are much to be desired. The compounds or groups of compounds produced are often characteristic of the species of organism used, in fact, this is one basis of bacteriological classification. As a result the usual practice is first to make specific tests for those substances whose presence is suspected. The next step is to discover by analysis whether all of the carbon is accounted for. If the amount not accounted for is larger than the error allowed by the methods of separation and analysis used a more systematic investigation is necessary. In this work it was not found feasible to ferment a large amount of medium and then make all the qualitative tests on its contents, for as soon as the flask has been opened it is subject to contamination from other bacteria and molds. Instead, the various fractions resulting from separation, such as volatile acids and non-volatile acids, from a number of flasks were carefully examined. Finally, when the various products were identified, and the methods of separation were developed, a qualitative examination was made for these products of a single flask.

At the time this work was inaugurated the price of xylose was so great that sucrose was used when it was desired to obtain a large amount of some product for closer examination and development of analytical methods. This substitution is justified by the fact that as a rule the same fermentation products are produced from sucrose or from xylose. The ratio of these products, however, may not be identical from different sugars. For final results analyses were carried out on both xylose and sucrose substrates.

## 2. *The Identification of the Products Formed from Xylose and Sucrose*

The medium was placed in a two-liter Erlenmeyer flask, closed with a cotton plug and sterilized one-half hour at 15 pounds pressure. After cooling to room temperature it was inoculated with 5 cc. of a 24 hour culture and incubated at 37°.

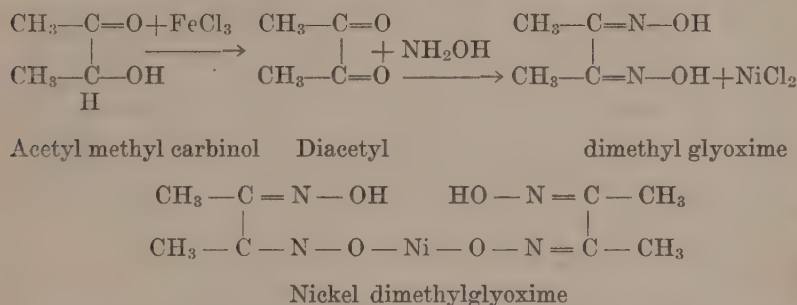
At the end of eight days the fermentation had apparently ceased, so the contents were subjected to investigation. The tests were carried out as follows:

*Xylose.* A 25 cc. portion was tested for unused xylose by means of the phloroglucinol method as described in the Official Methods of the A.O.A.O. This method is quantitative and is based on the transformation of the xylose to furfural when treated with 12 per cent HCl. The furfural is then determined by precipitating it as the furfural phloroglucide which can be filtered and weighed. No xylose remained unfermented.

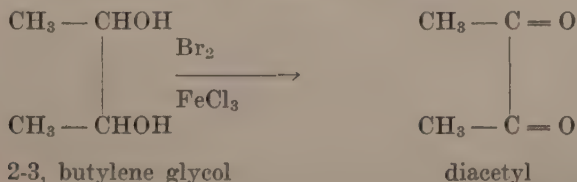
*Acetylmethyl carbinol and 2-3-butylene glycol.* The solution was tested for the presence of acetylmethyl carbinol and 2-3-butylene glycol by the specific test devised by Lemoigne (1920) and modified by Kluyver, Donker and Visser't Hooft (1925). Both products were found to be present, the 2-3-butylene glycol apparently predominating in amount. The test was carried out as follows: A 15 cc. sample of the neutralized solution was placed in a 500 cc. distilling flask, connected to a water cooled condenser and mixed with 5 cc. of a 45 per cent ferric chloride solution and 1 cc. of 0.5 N acetic acid. Heat was applied and 10 cc. slowly distilled into a

test tube. The distillate was mixed with 1 cc. of a 20 per cent water solution of hydroxylamine hydrochloride, 2 cc. of a 20 per cent solution of sodium acetate and about five drops of 10 per cent nickel chloride solution. The mixture was then boiled for a few minutes. A precipitate of fine red needles was formed if acetylmethyl carbinol was present in the original solution.

The test is caused by the oxidation, by ferric chloride, of the acetylmethyl carbinol to the easily volatilized diacetyl which is distilled off. The diacetyl reacts with the hydroxylamine to form dimethyl glyoxime, which in turn reacts with the nickel chloride to form the characteristic red insoluble nickel dimethyl glyoxime. These transformations can be symbolized as follows:



The ferric chloride oxidizes the acetylmethylcarbinol but not the 2-3-butylene glycol. Bromine, in the presence of iron salts, oxidizes 2-3-butylene glycol to diacetyl. The glycol can therefore be tested for in the residue from the acetylmethylcarbinol test. The glycol, having a low vapor pressure, is not appreciably lost when the diacetyl is distilled off. The test was carried out as follows. The residue in the distilling flask was mixed with 15 cc. of water, 2 cc. of bromine and 3 g. of solid sodium acetate and refluxed for 20 minutes on a water bath. The most satisfactory apparatus in which to carry out the bromine treatment was found to be a soil flask. This consists of an Erlenmeyer flask with a ground glass stopper in which is sealed a straight tube about 100 cm. long. The water jacket of a condenser can be attached to it to make a water cooled reflux condenser. After cooling to room temperature the solution was decanted from any liquid bromine remaining. The bromine in the solution was exactly neutralized by means of a saturated solution of sodium thiosulfate, using starch potassium iodide test papers to determine the neutralization point. The solution was then slowly distilled, 10 cc. were collected, neutralized to litmus with NaOH and tested for diacetyl as above.



*Alcohol.* The remainder of the solution was made distinctly alkaline to litmus with  $\text{Ca}(\text{OH})_2$ , filtered from excess  $\text{CaCO}_3$  and distilled, first at atmospheric pressure, then finally under reduced pressure (20 mm. at  $55^\circ\text{C}.$ ) until less than 50 cc. remained. The distillate was mixed with 50 g.  $\text{NaHSO}_3$  to hold back the acetylmethylcarbinol, and distilled until about 100 cc. remained. This distillate, which was slightly acid due to the presence of some  $\text{SO}_2$ , was made alkaline with  $\text{NaOH}$  and again distilled, using a short fractionating column. The distillation was continued until less than 100 cc. of solution remained in the distilling flask. The distillate, which was neutral to litmus, was mixed with about 25 g. of potassium dichromate and 50 cc. of concentrated sulfuric acid and then refluxed for 15 minutes. After cooling to room temperature the solution was transferred to a distilling flask and distilled until about 200 cc. remained. This residue was subjected to steam distillation until a 10 cc. fraction of the distillate collected in a test tube did not decolorize one drop of phenolphthalein in one drop of 0.1 N  $\text{NaOH}$ . The acid distillate was made up to one liter and a 50 cc. portion titrated with 0.0611 N  $\text{Ba}(\text{OH})_2$  using phenolphthalein as indicator. The amount required was 19.15 cc. A 400 cc. portion, equivalent to 153.2 cc. of 0.0611 N  $\text{Ba}(\text{OH})_2$ , was then subjected to a Duclaux distillation as modified by Knetemann. (See description in the section on quantitative analytical methods.) The results are given in table 5.

TABLE 5. *Distilling constants of volatile acid produced from alcohol*

No. of cc. distilled from 400 cc.	Cc. 0.0611 N $\text{Ba}(\text{OH})_2$ required	Pctg. acid in distillate	Pctg. acid for pure acetic (Knetemann)
50	12.8	8.3	8.2
100	13.6	17.2	16.8
150	14.3	26.6	26.1
200	15.2	36.5	36.1
250	16.3	47.2	46.9
300	18.0	58.9	59.1

The distilling constants, as shown in table 5, for the acid obtained by oxidation of the alcohol, agree very well with the distilling constants for pure acetic acid. Confirmation was obtained by neutralizing the remainder of the solution with  $\text{NaOH}$ , evaporating to dryness and preparing the p-toluide as described by Mulliken (1904, pp. 81-82). The p-toluide melted sharply at  $147^\circ$ . The melting points of the p-toluides of the volatile acids are given by Mulliken as follows:

Acetic acid	acet-p-toluide	m.p. $146^\circ\text{--}7^\circ$
Propionic acid	propion-p-toluide	m.p. $123.5^\circ\text{--}124.5^\circ$
n-Butyric acid	butyr-p-toluide	m.p. $72.5^\circ\text{--}73.5^\circ$

This proves ethyl alcohol to be the only volatile alcohol present in appreciable quantities in the fermentation mixture.



*Volatile Acids.* The residue from the removal of the neutral volatile products was acidified to Congo-Red paper with dilute  $\text{H}_2\text{SO}_4$  and subjected to steam distillation. The distillation was discontinued when a fraction of more than 5 cc. of distillate was required to decolorize one drop of phenolphthalein in one drop of 0.1N NaOH. The distillate, about 1500 cc., was made up to two liters and an aliquot portion titrated. It was found that 400 cc. were equivalent to 105 cc. of 0.0611 N  $\text{Ba}(\text{OH})_2$ . A 400 cc. portion was then subjected to a Knetemann distillation. The results are given in table 6.

TABLE 6. *Distillation constants of volatile acids*

Cc. distilled from 400 cc.	Cc. 0.0611 N $\text{Ba}(\text{OH})_2$ required	Pctg. acid in distillate	Pctg. acid for pure formic (Knetemann)	Pctg. acid pure acetic
50	7.5	7.1	4.7	8.2
100	8.2	14.9	10.0	16.8
150	8.4	22.9	16.0	26.1
200	9.2	31.7	22.6	36.1
250	10.1	41.3	30.5	46.9
300	11.5	52.3	40.1	59.1

The distilling constants in table 6 indicate that the volatile acids are largely formic and acetic, though the evidence is not conclusive since these results might conceivably be obtained by a mixture of formic with one of the higher volatile acids such as propionic or butyric. To clear up this point one-half of the remaining acid solution was neutralized with NaOH and evaporated completely to dryness. The salt obtained was tested for formates by dissolving part of it in water containing a little  $\text{Hg}_2\text{Cl}_2$  and boiling. A white precipitate of  $\text{Hg}_2\text{Cl}_2$  was obtained, which under the conditions used is a positive test for formic acid. A little of the salt was also boiled with  $\text{AgNO}_3$ . A black precipitate of metallic silver was obtained, showing the pressure of formates. The salt also reduced alkaline potassium permanganate. (Mulliken 1904, p. 83 and Allen, I., p. 486.) The results obtained in the manner described are satisfactory proof of the presence of formic acid. The remainder of the sodium salt was examined to determine the nature of the other volatile acid radicals by preparing the p-toluides as described above. After several recrystallizations a p-toluide was obtained which melted at  $148^\circ$ , showing the presence of acetic acid in the fermentation mixture. These results show that the only volatile acids present in appreciable amounts are formic acid and acetic acid.

*Non-Volatile Acids.* The residue from the steam distillation of the volatile acids was extracted continuously with ether for 72 hours in an apparatus described later. The ether extract was added to about 400 cc. of water and the ether removed by warming on a water bath. The solution was then boiled for a few minutes and titrated hot with 0.25 N  $\text{Ba}(\text{OH})_2$  using phenolphthalein as indicator. The solution, containing the barium salts of the non-volatile acids, was then evaporated on a water bath to a

volume of about 30 cc. It was then poured into 120 cc. of 95 per cent ethyl alcohol and allowed to stand for two days. The barium succinate is quite insoluble and precipitates out. Barium lactate is soluble under these conditions. The precipitate was filtered off and dissolved in about 50 cc. of water. A slight excess of  $\text{H}_2\text{SO}_4$  was added to precipitate the barium as  $\text{BaSO}_4$  and the solution was filtered and extracted for 48 hours with ether. The extract was evaporated to dryness and the crystals obtained were treated with p-toluidine in the manner described by Mulliken (p. 86). The melting point of the p-toluide obtained was  $256^\circ$ . The melting point given by Mulliken for succinic acid p-toluide is  $254.5^\circ$ - $255.5^\circ$ . The crystals of succinic acid when recrystallized from hot water, dried, and mixed with an authentic sample of succinic acid did not depress its melting point of  $186^\circ$ . These results prove the presence of succinic acid. The solution remaining after filtering off the barium succinate was tested for lactic acid by both the Uffelman (1909) and Fletcher Hopkins (1907) tests. Both tests were positive.

The Uffelman test was carried out as follows. A reagent was prepared by mixing 10 cc. of a 4 per cent solution of phenol with 20 cc. of water and adding one drop of 1 per cent  $\text{FeCl}_3$ . This forms a clear liquid of an amethyst color, which is turned yellow by a solution containing lactic acid. According to Uffelman, the test is sensitive to 1 part in 10,000 of lactic acid. This test is not specific for lactic acid, but is given also by tartaric, citric, malic and oxalic acids. However, the presence of none of these other acids is to be expected in a fermentation of this type.

The Fletcher Hopkins test was carried out in the following manner. A few drops of the suspected solution, 5 cc. of concentrated  $\text{H}_2\text{SO}_4$ , and a drop of a saturated  $\text{CaSO}_4$  solution were heated in a test tube placed in a water bath for two hours. The tube was cooled and 2 to 3 drops of a dilute solution of thiophen (50 drops in 100 cc. alcohol) were added. The presence of lactic acid was shown by the formation of a light cherry-red color when the tube was again heated on the water bath. The reaction is caused by the formation of acetaldehyde from the lactic acid. The color is the result of a product formed by reaction of the acetaldehyde with the thiophen.

Both these tests, while giving a good indication of the presence of lactic acid, are not specific, so the zinc salt was prepared. The alcoholic solution of the barium lactate was evaporated on the water bath to remove the alcohol and the barium lactate was dissolved in about 100 cc. of water. The barium was removed by adding the theoretical amount of 0.25 N  $\text{H}_2\text{SO}_4$  to form  $\text{BaSO}_4$ , which was filtered off. The solution of lactic acid was then boiled with an excess of zinc carbonate and the excess zinc carbonate filtered off. The solution was decolorized by boiling with a few grams of the decolorizing carbon known as Norit and allowed to evaporate at room temperature in the open air. After crystallization the salt was dried to constant weight over sulfuric acid and the percentage of moisture determined by heating for three hours at  $103^\circ$ - $5^\circ$ . It was found that the moisture was 13 per cent. The racemic zinc lactate has three molecules of water, or 18.17 per cent, while the optically active form contains two molecules of water, or 12.9 per cent. The salt when dissolved in water was found to be dextro rotary when examined in a polarimeter. This shows that the free acid has a levo rotation.

*Gaseous Products.* A small portion of the medium containing xylose was placed in an ordinary fermentation tube, sterilized and inoculated with 1 cc. of a 24 hour culture. After the evolution of gas had stopped, on the third day, the tube was filled with 25 per cent NaOH and shaken. The volume of gas decreased from 9.5 cc. to 4.5 cc. The gas remaining exploded in air when ignited. This indicates that the gaseous product was composed of carbondioxide and hydrogen in approximately equal parts by volume.

In examining the products formed from sucrose the fermentation and analysis were carried out in exactly the same way as described for xylose. The presence of unfermented sugar, however, was determined by the reducing power before and after inversion by hydrochloric acid, as determined by the Shaffer Hartman (1921) method.

### C. *The Relative Amounts of the Products Formed Under Aerobic and Anaerobic Conditions from Xylose and Sucrose*

1. *Introduction.* Before an exact study of the effect of environmental change on the amounts of various fermentation products formed can be made, it will be necessary to have exact methods of analysis for these products. If the study were limited to the quantitative determination of a few selected substances, such as carbon dioxide or unfermented xylose, the problem would be much simpler, for the analytical methods for these compounds are fairly precise. But with the analytical methods available at the present time an exact determination of all the various products which are present in a fermentation mixture, such as results from the action of *Aerobacter faeni* on sugar, is impossible.

In view of this fact, it was decided to make a study of the relative amounts of the various products formed under conditions differing in environment as much as possible. Many organisms are quite sensitive to changes in chemical or physical environment and will not grow except under conditions varying within narrow limits. *Aerobacter faeni*, however, is a facultative organism growing almost equally well under both aerobic and anaerobic conditions. This suggested a study of this environmental change on the relative amounts of the various products formed.

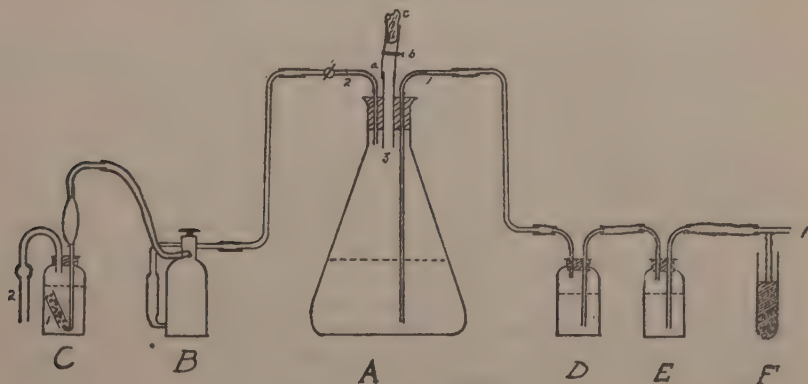


Fig. 1. Fermentation apparatus.



2. *Procedure.* All of the fermentations were carried out in the same type of apparatus. Aerobic conditions were established by passing air through the flask, and for anaerobic conditions hydrogen was used. The description of the apparatus is as follows: (See fig. 1.)

- A. The fermentation flask, a two-liter Erlenmeyer
  - 1. Gas inlet tube containing a plug of cotton at the outer end
  - 2. Gas outlet tube containing a glass stopcock
  - 3. Tube for introducing inoculum
    - (a) Thick walled rubber tubing
    - (b) Screw clamp
    - (c) Cotton plug
- B. Vanier absorption bottle containing 40 per cent KOH
- C. Absorption bottle containing 0.5 N KOH
  - 1. Short piece of glass tubing filled with beads
  - 2. Soda lime tube
- D, E. Gas washing bottles containing strong KOH
- F. Mercury trap
  - 1. Tube attached to gas source

Because of the decomposition of xylose at high temperatures in solutions containing salts, separate sterilization was employed when this sugar was used as substrate. The fermentation flask containing the medium was placed in the autoclave and sterilized 30 min. at 15 lbs. pressure. During the process the glass stopcock was closed and the screw clamp open. After sterilization the flask was connected to the remainder of the apparatus, with the screw clamp closed and the stopcock open. A stream of gas was bubbled through the medium while it cooled so that it would be saturated with the gas. After reaching the temperature of the incubator (37°C.), the flask was inoculated with 5 cc. of a 24 hour culture. On the next day, and each day following, the gas was shut off at (a) by means of a screw clamp, forcing it to escape through the mercury trap. The stopcock was then closed and the vanier bottle removed and weighed to determine the amount of CO<sub>2</sub> absorbed. To insure the presence of excess alkali the solution was renewed after 6 grams of CO<sub>2</sub> had been absorbed. After about 8 to 10 days, when practically all of the CO<sub>2</sub> had been evolved, the aeration was stopped. The flask was then cooled to room temperature and 10 cc. of 40 per cent CO<sub>2</sub>-free NaOH added to it. The reason for adding the strong alkali was to make the solution sufficiently alkaline to prevent any further bacterial action. It had one unexpected but very satisfactory result. A flocculent precipitate was formed, probably magnesium ammonium phosphate, which, when settling, carried down with it all of the suspended bacteria, leaving the solution perfectly clear. The solid material was filtered off and washed. The filtrate and washings were made up to two liters with CO<sub>2</sub>-free distilled water and analyzed by the methods described in the following paragraphs.



3. *Methods of Analysis Used.* Each different combination of products will require slightly different methods of approach in chemical analysis. In the following described methods only the substances which have been found present by the qualitative analysis are considered.

*Carbon dioxide.* The amount of  $\text{CO}_2$  produced in the fermentation was calculated after determining the amount absorbed in the absorption train, the amount present in the medium and the amount in the solids which had been filtered off. From this total was subtracted the amount originally present in the 10 g. of  $\text{CaCO}_3$ . The difference was the amount produced from the sugar. As stated previously, the amount of  $\text{CO}_2$  absorbed in the vanier bottle was determined by weight. The amount in the other gas absorption bottle was determined by titrating an aliquot portion with 0.5 N HCl, using phenolphthalein as indicator, after the addition of sufficient 2 N  $\text{BaCl}_2$  to precipitate the carbonate. By knowing the value of the blank the amount of  $\text{CO}_2$  absorbed could be calculated. The amount of  $\text{CO}_2$  present in the fermentation mixture was calculated from the determination of  $\text{CO}_2$  in a 200 cc. aliquot portion. The apparatus used was that described by Heck (1929). The same apparatus was used to determine the  $\text{CO}_2$  present in the solid material except that a vanier bottle was inserted between the absorption flask and the remainder of the apparatus to take care of the large amount of  $\text{CO}_2$  evolved.

*Acetylmethylcarbinol.* Two methods were used in determining the amount of this compound present in the mixture. The first method was the Kluyver, Donker and Visser't Hooft (1925) modification of Lemoigne's (1920) method as adapted for an analytical procedure by Wilson, Peterson and Fred (1927). The determination was carried out as follows. A 200 cc. aliquot portion of the fermentation mixture was made just acid to litmus with acetic and mixed with 20 cc. of 40 per cent  $\text{FeCl}_3$ . The solution was then slowly distilled until 100-125 cc. of distillate had been collected. The distillate was mixed with 10 cc. of 20 per cent hydroxylamine hydrochloride, 20 cc. of 20 per cent sodium acetate and 5 cc. of 10 per cent nickel chloride, and refluxed for 15 minutes. The precipitate of nickel dimethylglyoxime was filtered in a Gooch crucible, dried and weighed. Grams of nickel dimethylglyoxime  $\times 0.6097$  = grams of acetylmethylcarbinol.

Apparently no work has been done to determine the completeness of the oxidation of acetylmethylcarbinol to diacetyl by  $\text{FeCl}_3$ . Wilson, Peterson and Fred (1927) found that by starting with pure diacetyl only 70-75 per cent was recovered as the nickel dimethylglyoxime. Results obtained in the present investigation indicate that the values obtained for acetylmethylcarbinol by this method are consistently about 25 per cent lower than the values obtained by the second method. By comparing these results with those obtained for pure diacetyl mentioned in the foregoing, it would appear that the oxidation to diacetyl is complete, but that a consistent loss of about 25 per cent of the diacetyl occurs during the distillation or subsequent treatment.

The second method for the determination of acetylmethylcarbinol is based on the fact, discovered by Peehmann and Dahl (1890), that this substance reduces Fehling's solution. Kling (1906) found that the reaction was quantitative, the only product of oxidation being acetic acid. He reported that 1 gram of acetylmethylcarbinol was equivalent to 2.85 grams

of copper when oxidized by an alkaline solution of Cu O. Walpole (1911) used the method for the determination of acetylmethylcarbinol in fermentation mixtures, and reported that 1 cc. Fehling's solution = 2.48 Mg. of acetylmethylcarbinol. This indicates that one gram of the carbinol = 3.556 gm. Cu. Pederson and Breed (1928) employed the reduction method, but used a calculated value based on the equation  $\text{CH}_3\text{CHOHCOCH}_3 + \text{Cu O} = 2 \text{ Cu}_2 \text{ O}$ , which gives the ratio between cuprous oxide and acetylmethylcarbinol as 1 to 0.2767 or 1 gram acetylmethylcarbinol = 3.25 g. Cu. They considered their results too low. Since the reduction value of acetylmethylcarbinol was not known for the method of reduction used in the Shaffer Hartmann procedure, the value obtained by Kling was arbitrarily chosen. The method was adapted to the present problem by determining the reducing power of an aliquot portion of the fermentation mixture using the Shaffer Hartmann method and making a correction for the amount of sugar and formic acid present. It is possible that some acetylmethylcarbinol was lost by aeration during the fermentation.

*2-3-Butylene Glycol.* Attempts to determine 2-3-butylene glycol by oxidation to diacetyl with bromine gave very inconsistent results, confirming the experience of Donker (1926). The method finally adopted consisted in extracting the glycol and weighing it. The determination was carried out in the following manner.

A 500 cc. aliquot portion of the fermentation mixture was evaporated under reduced pressure to a volume of about 100 cc., keeping the temperature below 45°. The solution was then saturated with NaCl and extracted for 72 hours with ether in the apparatus described below. (See fig. 2.)

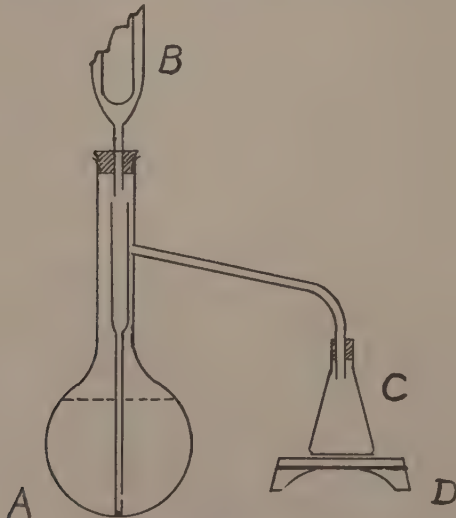


Fig. 2. Ether extraction apparatus.

- A. 250 cc. distilling flask.
- B. Hopkins condenser.
- C. 250 cc. Erlenmeyer flask.
- D. Electric hot plate.

After the extraction was completed the extract was mixed with about 20 grams of anhydrous sodium sulfate and allowed to stand over night. The salt was then filtered out and washed five or six times with ether which had been dried over sodium. The filtrate and washings were evaporated to a small volume and transferred to a weighing bottle. The remainder of the ether was evaporated and the glycol allowed to attain constant weight in a desiccator containing concentrated sulfuric acid. The amount of impurities in the glycol separated by this method is quite small, as was found when this fraction from a large amount of fermentation mixture was examined. However, it is necessary that the solution should be strongly alkaline as otherwise some acids will be extracted. When acetyl-methylcarbinol was found to be present in the fermentation mixture, the amount of it present in the glycol extract was determined and the correction applied. The determination was made by dissolving the glycol in water, making up to 100 cc. and examining an aliquot portion for reducing power.

*Sugar.* Xylose was determined in a 50 cc. portion of the mixture by the phloroglucinol method. Sucrose was calculated from the difference in reducing power before and after inversion. Inversion was accomplished by mixing 50 cc. of the mixture, which had been made just acid to litmus with HCl, with 5 cc. of Conc. HCl and allowing it to stand 24 hours at room temperature. The solution was then made just alkaline to litmus with strong KOH, made up to 100 cc. and an aliquot portion titrated by the Shaffer Hartmann method. In carrying out the reduction the method of heat control, using a manometer, which is described by Morrow (1927, pp. 199-200) was used.

*Ethyl Alcohol.* Most of the methods in use for the determination of small quantities of ethyl alcohol are based on its oxidation to acetic acid by means of  $K_2Cr_2O_7$  and  $H_2SO_4$ . The usual procedure is to distill off the alcohol from a neutral solution, treat the distillate with a mixture of potassium dichromate and sulfuric acid and distill with steam. The acetic acid in the distillate is then titrated with alkali. Dox and Lamb (1916) modified the method so as to determine the alcohol in a small sample of solution. Their modification consisted in removing the alcohol from a dilute alcoholic solution saturated with ammonium sulfate, by passing air through it. The alcohol vapor was absorbed in concentrated sulfuric acid. The acid containing the alcohol was then mixed with  $K_2Cr_2O_7$  and steam distilled as described above. Tomoda (1929) modified the method of Dox and Lamb by saturating the solution with sodium bisulfite instead of ammonium sulfate, making possible the determination of ethyl alcohol in the presence of volatile aldehydes and ketones. This method did not prove satisfactory in the present investigation because the very small sample (5 cc. out of 2000 cc.) introduced a large error.

The method finally adopted was as follows. Two hundred cubic centimeters of the fermentation mixture were mixed with 35 g. NaCl and slowly distilled, using a fractionating column to keep back the 2-3-butylene glycol. A few pieces of porous plate were used to insure even boiling without superheating. About 125-150 cc. of distillate were collected. The distillate was made up to a volume of 200 cc. with  $CO_2$ -free distilled water, and an aliquot portion examined for the amount of acetyl-methylcarbinol present. A 50 cc. portion of the distillate was then placed in a 150 cc. pressure bottle with 10 grams of  $K_2Cr_2O_7$  and 30 cc. of 50 per cent  $H_2SO_4$ . The



bottle was then placed in boiling water for 20 minutes. After cooling, the solution was subjected to steam distillation. Each 100 cc. portion of distillate as collected was titrated with 0.0611 N  $\text{Ba}(\text{OH})_2$ , using phenolphthalein as indicator. The distillation was discontinued when the 100 cc. portion of distillate required less than 0.5 cc. of alkali. This titration gave the total amount of acetic acid present. From this value was subtracted the amount of acetic acid resulting from the oxidation of the acetylmethylcarbinol. (1 Mol acetylmethylcarbinol = 2 Mols acetic acid.) The interference of acetylmethylcarbinol in this determination is a factor that has been disregarded or overlooked by many workers in this field.

*Volatile Acids.* As the result of the qualitative analysis, it was found that the volatile acids to be determined were formic and acetic. Any method for these acids can be no more exact than the method used for separating them from the non-volatile acids. There have been several methods suggested for the determination of formic acid based on its reducing power, such as that of Kelin (1906), in which the neutralized acid is titrated with  $\text{KMnO}_4$ , and the method of Fincke (1913) based on the reduction of  $\text{HgCl}_2$  to  $\text{Hg}_2\text{Cl}_2$ , which is filtered and weighed. These methods could not be used conveniently in the present investigation, due to the interference of acetylmethylcarbinol, which reduces both  $\text{KMnO}_4$  and  $\text{HgCl}_2$ .

The method adopted in this work was a combination of the modifications of the Duclaux distillation suggested by Knetemann (1928) and Virtanen and Pulkki (1928). The distillation constants and the method of manipulation were those of Knetemann, the method calculating the relative amounts of the volatile acids present was that described by Virtanen and Pulkki. The original articles should be consulted for the theoretical treatment and the essential details of apparatus and technique. The method is fairly precise for two volatile acids, but much less so for three.

The determination was carried out in the following manner. A 500 cc. portion of the alkaline fermentation mixture was evaporated to a volume of about 200 cc., made acid to Congo-Red paper with 25 per cent  $\text{H}_2\text{SO}_4$  and steam distilled. The indicator paper was left in the solution and if, as sometimes occurred, after a few minutes the paper became red, sufficient acid was run in through the steam inlet tube to turn the paper blue again. The distillate was collected in a two-liter flask. After 1200-1500 cc. had distilled over, at intervals the flask was removed and the distillate collected in a test tube containing one drop of 0.1 N alkali. When more than 5 cc. of distillate were required to decolorize the indicator, the distillation was stopped. The distillate was then gently boiled for 15 minutes under a water cooled reflux condenser at least 70 cm. long, fitted at the top with a soda-lime tube, to drive out any dissolved  $\text{CO}_2$ . The contents of the flask were allowed to cool in the apparatus and then made up to two liters with  $\text{CO}_2$ -free distilled water. A 50 cc. portion was then titrated with 0.0611 N  $\text{Ba}(\text{OH})_2$  to determine the total acidity, using phenolphthalein as indicator. A 400 cc. portion of the acid solution was placed in the distilling flask with 2.5 g. of granular pumice stone, and distilled at the rate of about 50 cc. in 12 minutes. Each 50 cc. portion as collected was titrated with 0.0611 N  $\text{Ba}(\text{OH})_2$ . The distillation values calculated for the pure volatile acids by Knetemann are given in table 7.



TABLE 7. *Distillation values of the pure volatile acids (Knetemann)*

Dis- tilled  from 400 cc.	Percentage of acid in distillate						
	Formic acid	Acetic acid	Propi- onic acid	Butyric acid	Valeric acid	Caproic acid	Caprylic acid
50	4.7	8.2	15.9	23.0	33.8	42.1	69.1
100	10.0	16.8	31.0	42.7	58.6	69.0	91.9
150	16.0	26.1	45.6	60.0	76.4	85.4	98.3
200	22.6	36.1	59.3	74.0	88.3	94.2	99.8
250	30.5	46.9	72.1	85.2	95.2	98.2	100.0
300	40.1	59.1	83.5	93.3	98.6	99.7	-----
350	53.7	73.9	93.3	98.3	99.8	100.0	-----

The amount of each acid present is calculated by means of the following equations:

$$A + B = Z$$

$$a/100 A + b/100 B = Z_1$$

where

A = no. cc. of acetic acid

B = no. cc. of formic acid

Z = total acidity of 400 cc. portion in terms of the alkali used

Z<sub>1</sub> = no. cc. of alkali required for 200 cc.

a = distillation value for 200 cc. pure acetic

b = distillation value for 200 cc. of pure formic

The distillation values obtained for each of the four fermentations are given in table 8.

TABLE 8. *The distillation values obtained for the mixtures of volatile acids produced by fermentation*

Cc.  Distilled	Xylose		Sucrose	
	Aerobic	Anaerobic	Aerobic	Anaerobic
50	6.2	11.7	6.7	7.5
100	12.8	23.0	12.5	15.4
150	19.7	34.1	18.8	23.6
200	27.0	44.7	25.9	32.1

By comparing these values with the values for the pure acids it is evident that the only series which does not lie between the values for formic and acetic acid is that obtained in the anaerobic xylose fermentation. The high values indicate the presence of some acid higher than acetic, and the characteristic odor of butyric acid was very evident. In this experiment the amount of butyric acid was calculated from the following equations:

$$\begin{aligned} A + B + C &= Z \\ a'/100 A + b'/100 B + c'/100 C &= Z'_1 \\ a/100 A + b/100 B + c/100 C &= Z_1 \end{aligned}$$

where

A = no. cc. of acetic acid  
 B = no. cc. of formic acid  
 C = no. cc. of butyric acid  
 Z = total acidity of 400 cc. portions in terms of the alkali used  
 $Z'_1$  = no. cc. alkali required for 100 cc. of distillate  
 $Z'$  = no. cc. alkali required for 200 cc. of distillate  
 $a'$  = distillation value for 100 cc. pure acetic  
 $b'$  = distillation value for 100 cc. pure formic  
 $c'$  = distillation value for 100 cc. pure butyric  
 $a, b, c$  = corresponding values for 200 cc.

*Non-Volatile Acids.* The residue from the steam distillation of the volatile acids was mixed with about 15 grams of  $\text{Na}_2\text{SO}_4$  and extracted with ether for 72 hours in the apparatus previously described (fig. 2). The ether extract was then mixed with 400 cc. of distilled water and the ether evaporated. The solution was then boiled for about 10 minutes and titrated, while hot, with 0.2487 N  $\text{Ba}(\text{OH})_2$  using phenolphthalein as indicator. The solution was then carefully evaporated to a volume of about 100 cc. and then mixed with 400 cc. of 95 per cent ethyl alcohol. After standing for two days the precipitate of barium succinate was filtered off in a weighed Gooch crucible, dried for an hour at  $100^\circ$ , cooled in a desiccator and weighed. One gram of barium succinate = 0.4657 grams of succinic acid. By knowing the total amount of  $\text{Ba}(\text{OH})_2$  required for the non-volatile acids, and the weight of barium succinate, the amount of lactic acid can be calculated by difference.

4. *Results of Analysis.* The results obtained in the analysis are given in table 9, which gives the amount of the substance produced in grams and the percentage yield based on the carbon content. The values obtained from each sugar under aerobic and anaerobic conditions should be compared rather than the values for xylose and sucrose. The reason for this is that the two xylose fermentations were run parallel as were the two sucrose fermentations. It will be noted that no butyric acid was found in the anaerobic sucrose fermentation while 4.4 per cent was obtained in the anaerobic xylose fermentation. This difference is believed to be due, not to an inherent difference in the two fermentations, but to a difference in technique used. In the xylose fermentation the medium was cooled while the hydrogen was passing through it, while in the sucrose fermentation the solution was cooled before being aerated with hydrogen.

TABLE 9. *The relative amounts of the products formed under aerobic and anaerobic conditions from xylose and sucrose*

Products	Xylose				Sucrose			
	Aerobic		Anaerobic		Aerobic		Anaerobic	
	Grams	Pctg.	Grams	Pctg.	Grams	Pctg.	Grams	Pctg.
CO <sub>2</sub>	12.10	41.1	7.78	26.5	16.439	53.5	10.550	34.2
2-3, Butylene glycol	2.11	14.1	2.73	18.2	3.024	19.2	3.331	21.0
Acetylmethylcarbinol	0.52	3.5	0.05	0.3	1.288	8.4	0.113	0.7
Ethyl alcohol	1.26	8.2	2.58	16.8	2.252	14.1	3.862	23.9
Formic acid	0.76	2.5	0.14	0.5	0.427	1.3	0.534	1.6
Acetic acid	0.48	2.4	1.05	5.2	0.205	1.0	1.357	6.4
Butyric acid	.....	0.0	0.65	4.4	.....	0.0	.....*	0.0
Lactic acid	0.23	1.1	2.53	12.6	0.455	2.2	2.858	13.6
Succinic acid	0.34	1.7	0.09	0.5	0.332	1.6	0.534	2.6
Sugar unused	0.00	0.0	0.00	0.0	0.095	0.5	0.000	0.0

\*This may have been caused by a slight difference in technique. The medium was aerated for about three hours after cooling instead of during the process of cooling.

The results in table 9 show that the relative amounts of certain of the products are different when formed under aerobic and anaerobic conditions. Some of the differences are not believed to be of special significance because of analytical difficulties and variations in individual cultures. The last point has been particularly emphasized by Pederson and Breed (1928). Among the significant variations the following may be noted. Regardless of whether the sugar fermented is xylose or sucrose, anaerobic conditions decrease the production of CO<sub>2</sub> and acetylmethylcarbinol, while the yields of 2-3, butylene glycol, ethyl alcohol, acetic acid and lactic acid are increased. The increase in production of lactic acid is especially striking.

It is apparent that when organisms of the type of *Aerobacter faeni* are used in the large scale production of 2-3, butylene glycol and lactic acid, anaerobic conditions will give much higher yields.

#### DISCUSSION

The fact that only the substances mentioned in the preceding sections have been identified does not preclude the possibility of other substances being present in very small amounts. Undoubtedly other chemicals are present, some, as end products of the fermentation, more, however, as intermediary compounds whose presence is transient. A study of the latter compounds is of utmost importance in explaining the mechanism of the fermentation process. However, a study of the mechanism of fermentation is beyond the scope of this investigation. It may be mentioned in passing that any theory as to mechanism to be acceptable must satisfactorily explain the variation in amounts of individual products with changes in environment such as has been found in the present investigation.

In connection with the qualitative analysis there are several points which should be subjected to further study. Walpole (1911) found that the 2-3, butylene glycol produced by *Bacillus lactis aerogenes* from sugar was a mixture of several isomeric modifications. His results led him to believe that the mixture was composed of 90 per cent of an optically inactive form whose diphenyl urethane derivative melted at 199.5°, but his experiments gave no indication whether this inactive form was the meso- or racemic-isomer. From the other 10 per cent of the mixture he obtained another inactive form which gave a diphenyl urethane with a melting point of 157°. Since his glycol preparations were optically active, apparently some of the optically active isomer was present. Boeseken and Cohen (1928) made a complete study of the configuration of the 2-3, butylene glycols. They examined a sample of the glycol produced by fermentation and found that it was composed of a large proportion of the meso-isomer and small amounts of the optically active and racemic forms. The nature of the 2-3, butylene glycol produced by *Aerobacter faeni* should be studied along similar lines. Another point to be cleared up is the optical activity of the acetylmethylcarbinol produced in this fermentation. A third problem to be investigated is the examination of a large amount of the lactic acid produced by *Aerobacter faeni* to discover whether or not the levo-isomer is the only one present.

In connection with the quantitative investigation, a determination which should be made is the ratio between the amounts of carbon dioxide and hydrogen, under both aerobic and anaerobic conditions. It was omitted from the present work because of the procedure which was used in maintaining anaerobic conditions.

#### SUMMARY

It has been shown that *Aerobacter faeni* grows well on a simple medium containing ammonium chloride as the sole source of nitrogen. A qualitative analysis has shown that the same products are formed from both xylose and sucrose. The products which have been identified are acetylmethylcarbinol, 2-3, butylene glycol, ethyl alcohol, formic acid, acetic acid, butyric acid, l-lactic acid, succinic acid, hydrogen and carbon dioxide. The relative amounts of these products (except hydrogen) produced under aerobic and anaerobic conditions have been studied. The results show that the amounts of certain products do depend on these conditions. It would seem that when organisms of the type of *Aerobacter faeni* are used in the large scale production of 2-3, butylene glycol or lactic acid, anaerobic conditions will give much higher yields.



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# THE EFFECT OF MANURES AND CROP RESIDUES ON NITROGEN CHANGES AND MICROORGANISMS IN THE SOIL

F. B. SMITH AND P. E. BROWN

*From the Laboratory of Soil Chemistry and Bacteriology, Iowa State College*

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Experiments have shown that the production of ammonia and nitrates may indicate the rate of decomposition of soil organic matter. It has been found also that for certain kinds of organic matter the amounts of ammonia and nitrates produced are correlated with the amount of carbon dioxide evolved, which is probably the most accurate measure of decomposition. However, with highly carbonaceous materials there may be a large production of carbon dioxide and a low production of nitrates. A determination of the nitrogen changes in a soil may, therefore, indicate the availability and hence the fertility value, at least of certain kinds of organic matter.

It is well known that such crop residues as straw and corn stalks decompose more slowly than leguminous residues. With the former the nitrate content of the soil may be reduced, the decrease being much greater than would be expected from differences in nitrification or in total nitrogen content of the organic matter. It has been concluded, therefore, that the assimilation of nitrates is stimulated to a greater extent by highly carbonaceous materials which may decompose more slowly in the soil than by materials of a narrow carbon-nitrogen ratio. The varying effects of different types of organic matter on soil may be caused by their influence on the nitrogen changes in the soil including not only nitrate accumulation and production but also assimilation.

In much of the work along this line, nitrate accumulation rather than nitrate production has been measured. Obviously nitrate accumulation alone cannot serve as a measure of decomposition as the amount of nitrates present at any given time is a resultant of nitrate production on the one hand, and on the other of assimilation, denitrification and losses by leaching. Similarly the production of nitrates alone will not show the rate of decomposition. The nitrifying power of the soil, the utilization or the nitrate-assimilating power of the soil, and the accumulation or the amount of nitrates present, must all be determined in order to get a more complete picture of the nitrogen changes. It is necessary that all these tests be made to determine the exact effect of any given treatment.

The purpose of this work was to correlate the nitrate-assimilating power of soils variously treated with manures and crop residues and the types of organisms developing in greater numbers with the treatments, with the nitrifying power of the soil and the accumulation of nitrates.

## EXPERIMENTAL

### *Methods of Procedure*

Dry virgin Carrington loam was treated with one per cent dry weight of various artificial farm manures, ordinary farmyard manure and certain residues such as oat straw, corn stalks and sweet clover tops and roots,

in four-gallon pots in the greenhouse. The materials were finely ground and thoroughly mixed with the soil. Each treatment was made in duplicate according to the outline in table 1. The moisture content of the soils was adjusted to approximately 50 per cent of the saturation capacity and maintained by frequent additions of distilled water. Samples for analysis were taken of each soil after one, four, nine and 13 weeks. The nitrate content of the soils, their nitrate-assimilating and nitrifying powers and the number and genera of molds were determined at each sampling. At the first sampling the numbers of bacteria developing upon nitrate agar and upon cellulose agar were determined. The nitrogen content of the manures and residues was determined by the Kjeldahl-Gunning method. The pentosans were determined by converting into furfural and precipitating with phloroglucide. While this method probably does not accurately determine pentosans, the results are comparable, and sufficient for the purposes of this experiment.

TABLE 1. *Outline of treatments and the analysis of materials*

Soil No.	Treatment	Percentage N. Kjeldahl-Gunning method	Percentage pentosans
1.	Check	-----	-----
2.	Farmyard manure	1.41	8.37
3.	Adco-straw manure*	1.91	7.81
4.	Adco-stalk manure*	2.07	8.95
5.	Ammonium sulfate-straw manure*	2.10	9.46
6.	Dry oat straw	0.96	19.73
7.	Corn stalks	0.60	22.52
8.	Sweet clover tops	2.03	15.04
9.	Sweet clover roots	1.74	12.91

\*The artificial manures were made by the Adco process, which is described in detail in Resch. Bul. 126, Iowa Agr. Exp. Sta.

Nitrates were determined colorimetrically by the phenol-disulfonic acid method. The nitrifying power of the soil was determined by the tumbler method according to Waksman (5), measuring the nitrifiability of the nitrogen contained in the manures as well as the effects of the treatments on the nitrifying power of the soil.

The nitrate-assimilating power of the soils was determined by measuring the utilization of nitrates in the soil in the presence of an excess of nitrates and two per cent of dextrose. Four 100-gram equivalents of dry soil from each pot were weighed into tumblers. To the soil in duplicate tumblers two grams of dextrose were added and thoroughly mixed with the soil and 30 milligrams of nitrogen were supplied by adding  $\text{KNO}_3$  in solution. Duplicate tumblers of each soil were not treated and served as checks. The moisture content of all the soils was adjusted to 30 per cent and the tumblers were kept at room temperature for four weeks. After incubation the nitrate content was determined. The figures for the nitrate-assimilating power were obtained by subtracting the amount present after incubation from the amount present in the non-treated soil after incubation plus the amount in the addition. That is, of course, assuming that nitrification may take place in the treated soils during incubation. The proof of this assump-

tion cannot be readily demonstrated in this experiment, but the results of other experiments not reported here indicate that nitrification does take place when only two grams of dextrose are added and the incubation period is as long as four weeks. If one assumes that no nitrification takes place in the treated soil and calculates the nitrate assimilating power accordingly, practically the same tendencies may be obtained as by this method. It is recognized that this method for determining the nitrate-assimilating power of the soil is not entirely satisfactory, but it is the most satisfactory method available at the present time.

The nitrate agar used had the following composition:

Dextrose .....	10.0 grams	CaCl <sub>2</sub> .....	0.02 grams
KNO <sub>3</sub> .....	1.0 grams	Agar .....	15.00 grams
K <sub>2</sub> HPO <sub>4</sub> .....	0.5 grams	Water .....	1 liter
MgSO <sub>4</sub> .....	0.5 grams	pH .....	6.4

The cellulose agar was made according to the directions given by McBeth (3). Waksman's synthetic acid agar was used for determining the numbers of molds. Numbers of organisms were determined by the plate method. Dilutions of one to one hundred thousand were poured for bacteria except on nitrate agar where a dilution of one to one thousand was used and of one to one thousand for molds, except for soils 8 and 9, where a dilution of one to one hundred thousand was poured. The plates were incubated at 25°C. for three days for mold counts and seven days to three weeks for the bacteria. In determining the genera of molds, use was made of the work of Gilman and Abbott (1) and of Thom and Church (4).

### *Effect of Manures and Crop Residues on Nitrogen Changes*

The results of the studies on the nitrogen changes in the soils at the first sampling are given in table 2.

TABLE 2. *The effect of manures and crop residues on nitrogen changes in the soil (after one week)*

Soil No.	Treatment	Mgm. nitrate N. per 100 grams of dry soil			
		Nitrifying power		Nitrate assimilating power	Nitrate content
		Soil alone	Soil+30 mgm. N. as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> +210 mgm. CaCO <sub>3</sub>		
1.	Check	1.89	9.07	19.38	0.78
2.	Farmyard manure	1.93	11.15	19.46	0.80
3.	Adeo-straw manure	2.56	11.15	18.24	1.02
4.	Adeo-stalk manure	2.11	14.92	17.70	1.02
5.	Ammonium sulfate-straw manure	1.87	11.20	18.53	0.94
6.	Oat straw	0	10.17	19.60	0
7.	Corn stalks	0	10.85	18.59	trace
8.	Sweet clover tops	1.67	13.40	24.02	1.67
9.	Sweet clover roots	1.51	16.00	20.40	1.51

When incubated alone the nitrifying power of the soil was the greatest in the soil treated with the Adco-straw manure. The nitrate assimilating power of the soil was reduced below that of the check and a higher accumulation of nitrates occurred. The nitrogen in this manure was more readily nitrified than that in any of the other manures. The sweet clover roots and the Adco-stalk manure stimulated the nitrifying power of the soil as shown when the test was made with ammonium sulfate and lime. The oat straw and corn stalks also stimulated nitrification, but no nitrates accumulated, although the nitrate-assimilating power was not appreciably affected. The largest assimilation of nitrates occurred in the soils treated with sweet clover tops and roots. However, the nitrifiability of these materials and the stimulation in nitrification which they brought about led to a very high accumulation of nitrates. The farmyard manure stimulated nitrification slightly, but it also increased the nitrate assimilation and hence there was no effect on the accumulation of nitrates in the soil.

The results obtained at the second sampling as shown in table 3 were similar to those just discussed. The ammonium sulfate-straw manure, oat straw and corn stalks depressed nitrification when tested in the soil alone or treated with ammonium sulfate and lime. The Adco-stalk manure had little effect on nitrification, but the Adco-stalk manure increased the process slightly. The farmyard manure and the sweet clover tops and roots brought about a marked increase in nitrification, the sweet clover tops and roots having a slightly greater effect. The sweet clover tops and roots contained relatively large amounts of easily nitrifiable nitrogen and hence led to a large accumulation of nitrates in the soils. Nitrate assimilation was again the greatest in the soils treated with the sweet clover tops and roots and was about equally stimulated by the corn stalks and oat straw. The manures all decreased nitrate assimilation at this sampling.

TABLE 3. *The effect of manures and crop residues on nitrogen changes in the soil (after four weeks)*

Soil No.	Treatment	Mgm. nitrate N. per 100 grams of dry soil			
		Nitrifying power		Nitrate assimilating power	Nitrate content
		Soil alone	Soil+30 mgm. N. as $(\text{NH}_4)_2\text{SO}_4$ +210mgm. $\text{CaCO}_3$		
1.	Check	1.74	14.60	18.72	0.73
2.	Farmyard manure	1.83	21.60	18.24	0.90
3.	Adco-straw manure	2.07	14.98	18.43	0.98
4.	Adco-stalk manure	1.83	14.55	18.00	0.77
5.	Ammonium sulfate-straw manure	1.66	13.90	18.16	0.70
6.	Oat straw	0	12.55	19.46	0
7.	Corn stalks	0	12.80	19.13	0
8.	Sweet clover tops	8.40	22.80	21.87	3.64
9.	Sweet clover roots	8.40	22.80	21.65	3.66



The Adeco manures stimulated nitrification slightly after nine weeks as shown in table 4, and the sweet clover tops and roots had a marked influence, whereas the farmyard manure and the ammonium sulfate-straw manure had some effect when tested in the soil alone, but showed none at all when tested with ammonium sulfate and lime. The oat straw and corn stalks still depressed nitrification with both tests. The manures all showed some availability for the nitrogen contained in them, the Adeco-straw manure having a greater availability than the other manures. The sweet clover residues showed a large amount of easily nitrifiable nitrogen, the tops more than the roots.

TABLE 4. *The effect of manures and crop residues on nitrogen changes in the soil (after nine weeks)*

Soil No.	Treatment	Mgm. nitrate N. per 100 grams of dry soil			
		Nitrifying power		Nitrate assimilating power	Nitrate content
		Soil alone	Soil+30 mgm. N. as $(\text{NH}_4)_2\text{SO}_4$ +210 mgm. $\text{CaCO}_3$		
1.	Check	4.36	36.40	18.36	3.51
2.	Farmyard manure	6.67	36.40	19.77	3.87
3.	Adeco-straw manure	7.04	38.10	17.23	4.34
4.	Adeco-stalk manure	6.20	39.05	17.05	4.00
5.	Ammonium sulfate-straw manure	5.40	36.40	17.99	3.20
6.	Oat straw	0.98	33.60	17.97	0
7.	Corn stalks	0.50	33.68	17.27	0
8.	Sweet clover tops	50.00	62.93	41.00	15.28
9.	Sweet clover roots	41.70	52.00	39.70	9.00

Nitrate assimilation was depressed by the oat straw and corn stalks at this sampling, but no nitrates had accumulated. The sweet clover tops and roots had a very great effect on the nitrate-assimilating power of the soil at this date. The nitrate content of the different soils varied somewhat, the largest accumulation occurring with the sweet clover tops, and second with the sweet clover roots. The Adeco-straw manure, the Adeco-stalk manure and the farmyard manure had slight stimulative effects, while the corn stalks, oat straw and ammonium sulfate-straw manure depressed the accumulation.

After 13 weeks, as shown in table 5, the detrimental effect of the oat straw on nitrification had almost disappeared, but it still persisted with the corn stalks. The manures and sweet clover tops and roots stimulated nitrification in much the same way as at the former samplings. The nitrate assimilation was greatly decreased in all the soils at this sampling, but there was again an increase over the check with all the treatments, the sweet clover tops and roots having the greatest effect. Nitrate accumulation was increased in all the soils except those receiving oat straw and corn

stalks. It was the greatest in the soils with the sweet clover tops and roots. Only traces of nitrates were found again, however, in the soils treated with the oat straw and corn stalks.

TABLE 5. *The effect of manures and crop residues on nitrogen changes in the soil (after 13 weeks)*

Soil No.	Treatment	Mgm. nitrate N. per 100 grams of dry soil			
		Nitrifying power		Nitrate assimilating power	Nitrate content
		Soil alone	Soil+30 mgm. N. as $(\text{NH}_4)_2\text{SO}_4$ +210 mgm. $\text{CaCO}_3$		
1.	Check	4.66	34.30	4.18	3.25
2.	Farmyard manure	6.60	38.92	4.71	3.23
3.	Adco-straw manure	8.75	41.25	4.75	5.63
4.	Adco-stalk manure	7.70	38.50	5.50	4.05
5.	Ammonium sulfate-straw manure	7.15	35.46	4.90	3.50
6.	Oat straw	3.07	34.50	7.02	trace
7.	Corn stalks	1.65	30.80	5.90	trace
8.	Sweet clover tops	38.60	63.10	10.85	28.67
9.	Sweet clover roots	26.75	54.00	9.00	21.17

*The Effect of Manures and Crop Residues on the Nitrate-Assimilating and Cellulose-Decomposing Bacteria*

The number of bacteria developing on cellulose agar and nitrate agar were determined as described above and the results are shown in table 6.

TABLE 6. *Effect of manures and crop residues on the number of bacteria developing on nitrate agar and cellulose agar*

Soil No.	Soil treatment	Number of bacteria per gram of dry soil	
		Nitrate agar	Cellulose agar
1.	Check	600	6,190,000
2.	Farmyard manure	2,400	7,370,000
3.	Adco-straw manure	9,960	16,500,000
4.	Adco-stalk manure	6,120	14,700,000
5.	Ammonium sulfate-straw manure	600	19,900,000
6.	Oat straw	49,920	20,600,000
7.	Corn stalks	5,760	20,500,000
8.	Sweet clover tops	720	25,800,000
9.	Sweet clover roots	60,000	27,800,000

The bacteria developing upon nitrate agar may be primarily cellulose decomposers, but they are referred to here as "nitrate assimilators." The results indicate that while the nitrate assimilators may be cellulose decomposers, all cellulose decomposers apparently are not nitrate assimilators. There was a very large stimulation in the development of the nitrate assimilators by the sweet clover roots and the oat straw. All the other materials increased the numbers of these organisms, but to a much less extent, except the ammonium sulfate-straw manure, which apparently had little effect. In general, the numbers of nitrate assimilators and cellulose decomposers, the nitrate assimilation, and the pentosan content of materials were roughly proportional and inversely proportional to nitrate accumulation except with the sweet clover residues where some other factor, probably the type of mold action, enters.

The numbers of organisms developing on the cellulose agar were more nearly the same for the various treatments and apparently more closely correlated with the nitrogen changes. The largest number of cellulose-decomposers developed in the soil treated with the sweet clover roots. Smaller increases were obtained in descending order as named with the sweet clover tops, the oat straw, the corn stalks, the ammonium sulfate-straw manure, the Adco-straw manure, the Adco-stalk manure and the farmyard manure, the latter showing only a slight increase over the check soil. These results as a whole indicate that cellulose-decomposing bacteria may be the chief nitrate-assimilating organisms in a soil.

*Effect of Manures and Crop Residues on the Number and Genera of Molds Developing in the Soil*

The numbers of the different genera of molds isolated from the soils variously treated for the several periods are presented in tables 7, 8, 9 and 10.

TABLE 7. *Effect of manures and crop residues on genera of molds in soil (after one week)*

Soil No.	Treatment	Number of molds per gram of moist soil						
		Absidia	Alter-naria	Asper-gillus	Mucor	Tricho-derma	Zygo-rhyn-chus	Peni-cil-lium
1.	Check	100	400	2,100	200	-----	-----	100
2.	Farm manure	-----	-----	2,000	800	-----	-----	-----
3.	Adco-straw manure	100	-----	3,100	400	-----	-----	-----
4.	Adco-stalk manure	100	100	2,500	1,300	-----	-----	-----
5.	Ammonium sulfate-straw manure	100	-----	2,300	300	-----	-----	-----
6.	Oat straw	-----	100	1,300	1,000	-----	100	-----
7.	Corn stalks	100	-----	900	1,400	100	-----	-----
8.	Sweet clover tops	-----	-----	900	22,900	-----	-----	-----
9.	Sweet clover roots	-----	-----	-----	25,000	-----	-----	-----





TABLE 9. *Effect of manures and crop residues on genera of molds in soil (after nine weeks)*

Soil No.	Treatment	Number of molds per gram of moist soil								
		Alter-naria	Asper-gillus	Conio-thyrium	Demat-ium	Mo-nilia	Mucor	Peni-cillium	Rhi-zopus	Tricho-derma
1.	Check	.....	3,400	800	.....	.....	.....	200	.....	600
2.	Farm manure	.....	4,400	2,000	.....	.....	.....	5,800	200	200
3.	Adco-straw manure	.....	2,800	.....	.....	.....	600	2,800	200	200
4.	Adco-stalk manure	.....	1,400	.....	.....	.....	600	800	.....	400
5.	Ammonium sulfate-straw manure	200	1,800	400	200	.....	.....	3,400	.....	200
6.	Oat straw	.....	8,600	200	.....	200	.....	400	.....	200
7.	Corn stalks	.....	2,400	.....	.....	.....	.....	1,800	200	.....
8.	Sweet clover tops	.....	20,000	.....	Overgrown with Rhizopus					
9.	Sweet clover roots	....	8,000	.....	Overgrown with Rhizopus					

TABLE 10. *Effect of manures and crop residues on genera of molds in soil (after thirteen weeks)*

Soil No.	Treatment	Number of molds per gram of moist soil									
		Acremonia	Aspergillus	Dematiaceae	Gliocladium	Fusarium	Penicillium	Rhizopus	Trichoderma	Sporotrichum	Zygothrychum
1.	Check	.....	3,200	.....	.....	600	1,200	200	1,200	400	.....
3.	Farm manure	200	8,800	.....	4,800	.....	.....	200	.....	.....	.....
3.	Adco-straw manure	400	7,000	.....	.....	200	600	.....	.....	.....	.....
4.	Adco-stalk manure	200	6,000	.....	.....	200	.....	.....	800	.....	.....
5.	Ammonium sulfate-straw manure	.....	7,200	.....	.....	.....	.....	.....	1,400	.....	200
6.	Oat straw	.....	20,600	2,600	.....	.....	600	.....	400	.....	.....
7.	Corn stalks	.....	12,000	.....	.....	.....	.....	.....	200	.....	.....
8.	Sweet clover tops	.....	372,000	.....	.....	.....	.....	398,000	134,000	.....	.....
9.	Sweet clover roots	.....	233,000	.....	.....	.....	.....	180,000	.....	.....	.....

At the first sampling the species of *Mucor* and *Aspergillus* were the most abundant. Especially large numbers of species of *Mucor* were found in the soils treated with sweet clover tops and roots. Martin (2) reported large numbers of species of *Mucor* in soils treated with sweet clover tops and roots. Every soil tested in this work, except those treated with sweet clover and corn stalks, contained many species of *Aspergillus*. *Zygorhynchus* and *Alternaria* were found in the soil treated with straw and *Trichoderma* was found in the soil treated with corn stalks. These three genera of molds are well known cellulose decomposers, moreover, species of *Trichoderma* and *Alternaria* were capable of utilizing 8.10 and 7.90 milligrams, respectively, of nitrate nitrogen from the soil in seven days.

At the second sampling species of *Mucor* were found in all soils except those treated with the sweet clover tops and roots, which were completely overgrown with *Rhizopus* to the exclusion of other genera. *Rhizopus* was found in all soils except the check. Species of *Aspergillus* were found in all soils in large numbers except in those treated with sweet clover tops and roots. The numbers of species of *Aspergillus* were the largest in the soil treated with the Adco-straw manure and second largest in the soil treated with farm manure. A great many species of *Trichoderma* were found in most of the soils at this sampling, the oat straw bringing about the greatest increase in the number of organisms of this genus. Only relatively few species of *Penicillium* were found at this sampling.

At the third sampling species of *Aspergillus*, *Penicillium*, *Rhizopus* and *Trichoderma* were found most frequently. Species of *Aspergillus* were found in large numbers in all the soils, the number being especially large in the soil treated with the sweet clover tops. The soils treated with the sweet clover tops and roots were overgrown with *Rhizopus* at this sampling.

At the last sampling very many genera were found. The species of *Aspergillus* were found in large numbers in practically all the soils and especially in the soils treated with the residues. The check soil contained the largest number of genera, being represented by *Aspergillus*, *Fusarium*, *Rhizopus*, *Penicillium*, *Sporotrichum* and *Trichoderma*. Many species of *Penicillium* were encountered in more soils at the third sampling than at this time. Species of *Rhizopus* were found in large numbers in the soil treated with sweet clover. Species of *Mucor*, which were found in the soil treated with the sweet clover residues at the first sampling, did not appear at this last sampling.

#### SUMMARY AND CONCLUSION

There has been considerable question among investigators whether the disappearance of nitrates from soils treated with such highly carbonaceous materials as straw is caused by a stimulated nitrate assimilation or a depressed nitrification. These results show that nitrate accumulation in the soil is prevented both by a depression in nitrate production and by a stimulation in nitrate assimilation when various organic materials are applied to the soil. The sweet clover tops and roots stimulated the nitrate-assimilating power of the soil to a greater extent than did the straw and corn stalks, yet more nitrates accumulated in the soils treated with these residues than in the soils treated with the straw and corn stalks. Undoubtedly, this, in part, is caused by the higher nitrogen content of the legumi-

nous residues and to their more rapid nitrification. It is also due, however, to the stimulation in the development of large numbers of *Mucor* and *Rhizopus* soon after treatment. These organisms do not decompose cellulose nor assimilate nitrates, but are large producers of ammonia, thus increasing nitrate production and accumulation. The straw and corn stalks, rich in cellulose and pentosans, stimulated the development of *Aspergillus*, *Trichoderma*, *Alternaria* and other cellulose decomposers. These organisms are also heavy feeders on ammonia and nitrates, thus depressing nitrate production, stimulating nitrate assimilation and preventing an accumulation in the soil. These results would also seem to indicate the occurrence of a nitrate-assimilating flora in the soil, and that the cellulose decomposers may be the principal nitrate consumers.

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# THE ISOMERIC FORMS OF LACTIC ACID PRODUCED IN MILK BY SOME OF THE ANAEROBES

R. V. HUSSONG AND B. W. HAMMER

*From the Section of Dairy Industry, Iowa Agricultural Experiment Station*

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The changes brought about in milk through the growth of microorganisms commonly involve the formation of lactic acid. This is produced not only by the organisms whose outstanding character when grown in milk is the elaboration of lactic acid, for example the members of the *Streptococcus lactis* group and some of the species of the genus *Lactobacillus*, but also by organisms whose conspicuous effect is the formation of one or more other compounds so that the lactic acid production is over-shadowed. Certain organisms belonging to the genus *Clostridium* are included in the latter group.

## OBJECT

The work herein reported was carried out to determine the isomeric form of lactic acid produced in milk by cultures of anaerobes belonging to two types that are comparatively common in dairy products, *Clostridium welchii* and *Clostridium butyricum*. It involved (a) the preparation of zinc salts from the residues remaining when milk cultures were steam-distilled for the purpose of obtaining the volatile acids, and (b) the examination of these salts for the percentage of water of crystallization, the percentage of ZnO, and in case the salt was active, the direction of the rotation.

## HISTORICAL

Schattenfroh and Grassberger (4) studied three species of anaerobic bacteria. The one motile species, which did not liquefy gelatin, apparently formed in milk small amounts of inactive lactic acid in addition to butyric acid. The two non-motile species produced large amounts of *d*-lactic acid in addition to butyric acid. One of the cultures investigated produced succinic acid as well as butyric acid. In a later paper Schattenfroh and Grassberger (5) pointed out that the ratio between lactic and butyric acids produced by these organisms was variable. The motile species produced *d*-lactic and butyric acids from dextrose, and probably also from sucrose and starch. It was not unusual for the amount of *d*-lactic acid to exceed that of butyric acid. The non-motile organisms when grown in milk produced nearly equal amounts of *d*-lactic and butyric acids although great irregularities were not unusual. In lactose bouillon there was more *d*-lactic acid formed than butyric acid. Glucose, sucrose and starch were also fermented to *d*-lactic and butyric acids.

Esty (2) noted that *C. welchii*, when grown in milk, formed lactic acid in addition to butyric acid.

Parsons and Sturges (3), in a study of the metabolism of *Clostridium putrefaciens*, found that non-volatile acids were not formed in meat medium.

Weigmann (6) stated that the non-motile species of anaerobic butyric acid bacteria curdle milk by forming lactic and butyric acids, and that the motile type forms more butyric acid than lactic acid.

#### METHODS

Thirteen hundred ml. lots of skimmed milk were sterilized in two-liter Erlenmeyer flasks and, after being rapidly cooled, each was inoculated with one of the cultures to be studied. The freshly sterilized milk provided good conditions for growth, and seals were not necessary. After incubating for one week at 37°C., the contents of a flask were transferred to a distillation flask, 60 ml. 1 N H<sub>2</sub>SO<sub>4</sub> added and a steam-distillation carried out; one liter of distillate was collected. Following cooling, the residue remaining was filtered. The whey obtained was evaporated to a small volume and then mixed with plaster of Paris to take up the remaining water.

The whey residue was put into a thimble and extracted with ether for about 15 hours. The ether extract was transferred to a beaker, the ether removed by evaporation, water added and then an excess of ZnCO<sub>3</sub>. After boiling with charcoal, the fat, ZnCO<sub>3</sub>, charcoal, etc., were filtered off and the zinc salt secured by concentration and crystallization; the crystallization was as complete as possible. The salt obtained was recrystallized at least once, dried, finely ground and dried at room temperature to practically constant weight. The percentage of water of crystallization was calculated from the loss on heating to constant weight at from 108 to 110°C. and the percentage of ZnO from the weight after burning in a furnace. When the water of crystallization indicated an optically active salt the direction of the rotation was determined.

#### EXPERIMENTAL

A zinc salt of the non-volatile acid produced was secured with ten cultures of *C. welchii* and with two cultures of *C. butyricum*; with a number of other *C. butyricum* cultures the amount of non-volatile acid present in the quantity of milk fermented by each culture was too small to yield sufficient zinc salt for satisfactory purification. One of the *C. welchii* cultures (designated A) was secured from Dr. I. C. Hall and was found by him to belong to Type 2, while the other nine were isolated from milk or cream; cultures C and J belong to Type 2; culture G to Type 3; and cultures B, D, E, F, H, I to Type 4. The *C. butyricum* cultures were isolated from milk or cream; they agreed with the description of this organism as given by Bergey (1) except in the action on sucrose.

All of the cultures studied grew well when inoculated into freshly sterilized milk in rather deep layers and readily produced gas and definite coagulation although the curd formed by the *C. welchii* cultures was usually much firmer than that formed by the *C. butyricum* cultures.

Data illustrating the volatile acid production in milk of each of the two species are presented in table 1; the incubation was one week at 37°C. With the six *C. welchii* cultures the volatile acids present in the first liter

TABLE 1. Volatile acid production in milk of *C. welchii* and *C. butyricum* (incubated one week at 37°C.)

Organisms	Culture designations	ml. 0.1 N NaOH required to neutralize the volatile acids in the first liter of distillate steam-distilled from 1300 ml. of fermented milk
<i>C. welchii</i>	A	116.4
<i>C. welchii</i>	B	89.2
<i>C. welchii</i>	C	57.3
<i>C. welchii</i>	G	47.9
<i>C. welchii</i>	I	86.1
<i>C. welchii</i>	J	42.5
<i>C. butyricum</i>	K	299.0
<i>C. butyricum</i>	L	194.9
<i>C. butyricum</i>	M	202.2

of distillate secured by steam-distilling the 1300 ml. of fermented milk required from 42.5 to 116.4 ml. of 0.1 N NaOH for their neutralization, with only one culture requiring over 100.0 ml. of 0.1 N NaOH, while with the three *C. butyricum* cultures corresponding values ranged from 194.9 to 299.0 ml. of 0.1 N NaOH. These results show that along with the firmer curd produced in milk by *C. welchii* than by *C. butyricum* there was a lower volatile acid production.

Table 2 gives the data secured on the zinc salts prepared from the non-volatile acids formed in milk fermented by *C. welchii* or by *C. butyricum*. The values for the percentage of H<sub>2</sub>O and the percentage of ZnO in the

TABLE 2. Data secured on zinc salts of the non-volatile acids produced in milk by *C. welchii* and *C. butyricum* (incubated one week at 37°C.)

Organism	Culture designation	Percentage H <sub>2</sub> O in Zn salt			Rotation of Zn salt	Percentage ZnO in the moisture free Zn salt
		A	B	Av.		
<i>C. welchii</i>	A	16.90	16.76	16.83		33.60
<i>C. welchii</i>	B	16.24	16.13	16.185		34.03
<i>C. welchii</i> 1st trial	C	13.66	13.48	13.57	1	33.89
<i>C. welchii</i> 2nd trial	C	13.76	13.69	13.725	1	33.51
<i>C. welchii</i>	D	12.88	12.96	12.92	1	33.48
<i>C. welchii</i>	E	13.22	13.26	13.24	1	33.03
<i>C. welchii</i>	F	13.90	13.82	13.86	1	33.30
<i>C. welchii</i>	G	13.18	13.15	13.165	1	33.45
<i>C. welchii</i>	H	13.17	13.04	13.105	1	33.31
<i>C. welchii</i>	I	13.07	13.27	13.17	1	33.54
<i>C. welchii</i>	J	12.88	12.88	12.88	1	
<i>C. butyricum</i>	K	18.30	18.38	18.34		34.06
<i>C. butyricum</i>	L	18.42	18.20	18.31		33.45
						33.62

salts indicate that the salts were Zn lactate; in general, the ZnO values which differed the most from the theoretical were secured on comparatively small amounts of salt. The values for the percentage of H<sub>2</sub>O indicate that with the *C. welchii* cultures the lactic acid produced was active or a mixture of active and inactive. The percentage of H<sub>2</sub>O in the Zn salts agreed very well with the theoretical for the salt of active acid with two cultures (D and J), the values were slightly higher with six cultures, which may have been caused by the presence of some free moisture or small amounts of inactive acid, while considerable inactive acid was undoubtedly present with two cultures (A and B). With the salt preparations from the eight cultures producing entirely or largely active acid the rotation was *l*, which indicates that the free acid was of the *d* type. The data presented show that with the two *C. butyricum* cultures the lactic acid was inactive. As previously stated, other *C. butyricum* cultures were investigated, but the amounts of non-volatile acid present were too small to yield quantities of zinc salt that were satisfactory for purification; an impure salt secured from milk fermented by one of these cultures showed water of crystallization and ZnO values that indicate inactive lactic acid, although these differed from the theoretical values to an extent suggesting considerable impurity.

#### SUMMARY

A number of cultures of *C. welchii*, when grown in milk produced lactic acid of the *d* type or a mixture of active and inactive acids. Two cultures of *C. butyricum*, when grown in milk, produced inactive lactic acid; a number of others yielded, from 1300 ml. of milk incubated one week at 37°C., such small amounts of zinc salt of the non-volatile acid that satisfactory purification was impossible. *C. welchii* produced smaller quantities of volatile acid in milk than *C. butyricum*.

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## ACIDITY AND COLOR CHANGES IN TOMATOES UNDER VARIOUS STORAGE TEMPERATURES

E. S. HABER

*From the Section of Vegetable Crops, Iowa Agricultural Experiment Station*

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Although tomatoes cannot be stored for long periods of time it is often desirable and necessary to hold them for several days or even several weeks. Many tomatoes are picked green for shipping purposes, especially from the southern states and Mexico. These are shipped in refrigerator cars and stored by wholesalers and retailers until they have ripened or until they can be disposed of to the retail trade. Very little has been published with regard to suitable storage conditions for ripening green fruits or holding ripe fruits. A great deal of work has been done with regard to storage conditions for apples and potatoes, but the same conditions are not suitable for tomatoes.

Duggar (2) studied the development of pigment in the tomato, but his work was concerned principally with development at high temperatures. He found that the red pigment, lycopersicin, is partially or completely suppressed when green fruits are ripened at a temperature of 30°C. or above; instead of the red pigment a yellow-orange or orange-red color was produced. The factors for reddening are not destroyed by high temperatures and a return of the fruit to favorable conditions permits pigmentation. At high temperatures, decreased acidity accompanied lycopersicin suppression. Experiments carried on by Diehl (1) with the varieties, Aeme, Globe and Stone tomatoes fully developed but still green indicated that they were not injured when held for one day at temperatures of 30° and 31°F. Tomatoes held at 50°F. ripened gradually in about nine days, while at 70°F. they ripened in five to seven days. Green tomatoes exposed to a temperature of 32°F. for 14, 20 and 30 days failed to ripen and quickly broke down when exposed to room temperature. Harvey and Wright (3) found that in general, tomatoes in storage or at rest will undergo a lower temperature than in transit because they can be undercooled to a considerable extent without freezing. Sando (5) reports an increase in moisture, acids and sugars and a decrease in solids, total nitrogen, starch, pentosans, crude fiber and ash during the ripening period. The effect of lack of ventilation on ripening was to increase the acid content approximately 138 per cent over that of vine ripened fruit.

This paper presents an account of some investigations pertaining principally to the changes in acid concentration of tomato fruits in storage at several temperatures. It includes measurements of the total acid as well as the H-ion concentration and observations as to the coloring or ripening of the fruits at several temperatures; also the rate of coloring or ripening at room temperature when removed from storage.

## MATERIAL AND METHODS

Tomatoes used in these experiments were grown in the college greenhouse. The tomatoes were pruned to a single stem, allowing seven to nine clusters to develop before topping. In the fall of 1929 both the Grand Rapids and Globe varieties were used for storage, but only the Globe variety was used in the spring and fall of 1930. The Globe variety is one of the principal forcing varieties used by greenhouse men in the northern states. The fall crop of 1929 was stored at temperatures of 36°F., 50°F. and 70°F. These storage temperatures did not vary more than two degrees except the 70°F. storage temperature, which occasionally fluctuated as much as five degrees. The relative humidity at 36° and 40°F. was 75 to 80, about 70 in the 50°F. storage and about 60 in the 70°F storage.

The tomatoes were placed in storage after sorting and grading within an hour from removal from the vines. Ripe fruits and green mature fruits were stored. "Green mature" is a term used by Rosa (4) and refers to fruits which are fully developed but still green or greenish yellow. Also, the color or condition of the abscission zone of the fruit stem may be taken as an indication of maturity.

Two samples of ten fruits each were used for analysis. Differences were noted in the total acid and pH value of individual fruits, but a composite of ten fruits checked fairly accurately if the fruits in each lot were of nearly uniform size and of the same degree of ripeness. Medium sized fruits were used as much as possible, although Sando (5) found that ripening proceeded at a uniform rate regardless of size and that maturity depended on age, not size. A composite sample of the juice of the fruits was made by squeezing individual fruits through cheese cloth and then filtering the total juice through filter paper in a Buechner funnel. Ten cc. of juice were titrated against N/10 KOH, using phenolphthalein as an indicator.

A portion of the juice was used for pH determinations on the 1930 fall crop of tomatoes employed for the storage experiments. The pH values were determined by the quinhydrone method. The pH values were also expressed as arithmetical "active acidity units" according to Wherry (6) in order to have some means for grasping the relative degrees of acidity.

## EXPERIMENTAL DATA AND RESULTS

Although Sando's (5) results show that maturity depends on age rather than size, the writer thought that the position of the fruit on the vine might influence the acid content.

Table 1 gives the results with large and small fruits, both ripe and "green mature," from the lower and upper parts of the vines. Fruits from the lower three clusters were included in one group and fruits from clusters five, six and seven in another group. No consistent differences in either total acidity or pH were noted. Evidently the acidity of the fruits is not affected appreciably by size or position on the vine. Ten fruits each were used to make the composite samples.

Tables 2 to 13, inclusive, give the acid changes as well as the color changes occurring in fruits when held at the various storage temperatures.

The mature green tomatoes contain more acid than ripe fruits, but the decrease in acidity is much more rapid in the green than in the ripe fruits in storage. This is especially true of tomatoes stored at 70°F. At the lower temperatures, 36° or 40°F., the acid changes in the green mature fruits are rather slow and do not accompany color changes or ripening. Very little color develops in five to six weeks time, while the decreases in acidity are as great, or nearly as great, as in "mature green" fruits held at 50°F. The same fruits held at 50°F. were ripe within four weeks with no less loss in acid than at 36° or 40°F. The green mature fruits at 40°F. developed very little color, but remained firm. These fruits when placed at room temperature, after holding at this temperature two weeks or longer, failed to ripen properly and broke down quickly.

Ripe fruits held at the lowest temperature decreased in total acid at about the same rate as those held at 50°F., but much slower than those held at 70°F. After the second week in storage the ripe red color gradually became a little lighter in color and was an orange-red at the end of five to six weeks. These ripe fruits were firm, but broke down rather rapidly when placed at room temperatures. Acidity changes at 40°F. were much more rapid in ripe fruits than in the green mature fruits held at the same temperature.

Tomatoes held at 50°F., either ripe or green mature, kept much better than fruits held at the lower or higher temperatures. Ripe fruits remained in good condition for at least five weeks and when placed at room temperature remained fairly firm for four or five days. Green mature fruits slowly ripened at this temperature, although the color was a shade lighter than vine-ripened fruits. The storage-ripened fruits remained firm at least five days when placed at room temperature and the flavor of either ripe or green mature fruits was satisfactory.

Green mature fruits held at 70°F. ripened in five to seven days and were dead ripe in ten days. Ripe fruits usually stood up well for five to seven days, but shriveling usually occurred in two weeks to three weeks time. The acid decreased rapidly with both mature and immature fruits. Loss of acid occurred much more rapidly at this temperature. Some fruits would remain firm with little or no shriveling for three or four weeks, but were rather flat in taste.

Daily tests of acidity were also run for seven days and the data are presented in tables 14, 15 and 16. The fruits picked for these tests were from vines which had produced most of their crop and the fruits were a trifle lower in acidity than fruits picked from the same vines earlier in the life of the plant. These plants had passed the period of profitable production and this may account for a slight decline in acidity. At 36°F. little change in total acidity or pH is found during the first week of the storage period, while at 50°F. these changes are slightly greater. Green mature fruits at 50°F. show greater loss in acid than ripe fruits and are about one-half ripe in that length of time. At 70°F. the acidity changes and changes in pH values are much greater and green mature fruits are fully ripened in that length of time.

Loss in weight was also recorded at 40°, 50° and 70°F. The same fruits were not used as those used for acidity tests, since the handling in weighing each week might have caused slight bruises, which would impair



the keeping qualities. The losses in weight are presented in table 17. Green mature fruits lose weight more rapidly than ripe fruits, especially at the beginning of the storage period. The least weight loss occurred at 40°F. and the most at 70°F. Ripe fruits stored at 50°F. lost very little more weight than the same fruits at 40°F., while green mature fruits lost weight much more rapidly at 50° than at 40°F.

### SUMMARY AND CONCLUSIONS

Low storage temperatures commonly recommended for many vegetables apparently are not satisfactory with tomatoes; 50°F. is much more satisfactory than 36° or 40°F. Changes in total acidity and pH are not much greater at the former temperature than at the latter. Green mature fruits ripen very slowly at 50°F. and are palatable and stand up well at room temperatures after several weeks storage at this temperature. Ripe fruits also stand up well on removal to room temperature and there is little change in color at the end of five weeks in storage.

Green mature fruits stored at 36° or 40°F. fail to ripen properly in storage and when held at these temperatures for two weeks fail to ripen properly at room temperatures. Although the fruits are firm, the color is unattractive. With prolonged storage, four to six weeks, they break down quickly when placed at room temperature. Ripe fruits at this temperature change color from a ripe red color to an orange-red and appear watery and not very firm. They break down quickly when placed at room temperatures after two weeks in this storage.

Tomatoes stored at 70°F. lose weight and acid very fast, and green mature fruits ripen within a week or less at this temperature. Shriveling or wrinkling occurs in both ripe and green mature fruits in a short time. Some specimens remain firm without shriveling for five and six weeks, but taste flat, probably because the acidity has decreased very much.

Fifty degrees F. is a much better temperature than 36° or 40° F., at which to store either ripe or green fruits. Although less weight is lost and there is less change in acid content, the fruits fail to ripen as well at the lower temperatures. Unless quick ripening of green fruits is desired, 70°F. is too high a temperature for storage for more than five days.

TABLE 1. *Acidity of large and small fruits from upper and lower clusters*

	Total acid	pH
Large ripe fruits from 5th to 7th cluster	8.0	4.35
Small ripe fruits from 5th to 7th cluster	8.2	4.33
Large ripe fruits from 1st to 3rd cluster	8.3	4.33
Small ripe fruits from 1st to 3rd cluster	7.9	4.35
Large green mature fruits from 5th to 7th cluster	14.1	4.05
Small green mature fruits from 5th to 7th cluster	13.8	4.05
Large green mature fruits from 1st to 3rd cluster	13.7	4.00
Small green mature fruits from 1st to 3rd cluster	14.0	4.03



TABLE 2. *Acidity changes in Grand Rapids tomatoes stored at 36°F., fall crop 1929*

Length of storage period	Ripe		Green mature	
	Total acid	Color change	Total acid	Color change
7 days	14.4	red ripe	17.5	yellowish green
14 days	14.4	red ripe	14.6	slight pink blush
21 days	13.9	red ripe	14.2	slight increase in color
28 days	11.5	lighter red	12.7	slight increase in color
35 days	10.5	lighter red	11.6	slight increase in color
42 days	8.1	orange red		

TABLE 3. *Acidity changes in Grand Rapids tomatoes stored at 50°F., fall crop 1929*

Length of storage period	Ripe		Green mature	
	Total acid	Color change	Total acid	Color change
7 days	14.3	red ripe	17.6	yellowish green
14 days	13.0	red ripe	15.8	one-half ripe
21 days	12.1	red ripe	15.1	ripe
28 days	11.4	red ripe	13.8	ripe
35 days	10.5	red ripe	11.0	ripe

TABLE 4. *Acidity changes in Grand Rapids tomatoes stored at 70 F., fall crop 1929*

Length of storage period	Ripe		Green mature	
	Total acid	Color change	Total acid	Color change
7 days	10.6	red ripe	12.6	ripe
14 days	9.8	dead ripe	9.5	ripe
21 days	9.1	dead ripe	9.4	dead ripe
28 days	8.5	slight shriveling	8.7	slight shriveling
35 days	6.9	shriveling and spoilage	8.2	spoilage

TABLE 5. *Acidity changes in Globe tomatoes stored at 36°F., fall crop 1929*

Ripe			Green mature	
Length of storage period	Total acid	Color change	Total acid	Color change
7 days	11.5	red ripe	14.5	greenish yellow
14 days	11.4	red ripe	14.4	slight pink blush
21 days	9.5	lighter red	14.2	slight increase in color
28 days	8.0	lighter red	12.7	slight increase in color
35 days	7.5	lighter red	12.2	slight increase in color
42 days	5.8	still lighter	12.3	slight increase in color but not half ripe

TABLE 6. *Acidity changes in Globe tomatoes stored at 50°F., fall crop 1929*

Ripe			Green mature	
Length of storage period	Total acid	Color change	Total acid	Color change
7 days	12.6	red ripe	18.5	yellowish green
14 days	11.5	red ripe	14.7	considerable pink showing
21 days	10.7	red ripe	13.6	one-half ripe
28 days	7.6	red ripe	11.5	ripe
35 days	7.2	red ripe	9.5	ripe

TABLE 7. *Acidity changes in Globe tomatoes stored at 70°F., fall crop 1929*

Ripe			Green mature	
Length of storage period	Total acid	Color change	Total acid	Color change
7 days	8.6	red ripe	12.6	ripe
14 days	8.2	dead ripe	9.4	red ripe
21 days	6.3	dead ripe	8.6	dead ripe
28 days	4.0	mold and rot developing	7.0	some spoilage

TABLE 8. *Acidity changes in Globe tomatoes stored at 36°F., spring crop 1930*

Length of storage period	Ripe		Green mature	
	Total acid	Color change	Total acid	Color change
7 days	14.3	red ripe	17.5	greenish yellow
14 days	14.1	red ripe	15.5	slight pink blush
21 days	10.1	lighter red	14.1	slight increase in color
28 days	9.8	lighter red	12.7	slight increase in color
35 days	8.1	lighter red	12.9	slight increase in color
42 days	6.8	still lighter orange red	11.2	slight increase in color but not half ripe

TABLE 9. *Acidity changes in Globe tomatoes stored at 50°F., spring crop 1930*

Length of storage period	Ripe		Green mature	
	Total acid	Color change	Total acid	Color change
7 days	14.3	red ripe	17.1	yellowish green
14 days	10.5	red ripe	15.5	considerable pink showing
21 days	10.3	red ripe	15.3	one-half ripe
28 days	8.1	red ripe	11.8	ripe
35 days	7.8	red ripe	10.5	ripe
42 days	7.4	red ripe	9.2	ripe

TABLE 10. *Acidity changes in Globe tomatoes stored at 70°F., spring crop 1930*

Length of storage period	Ripe		Green mature	
	Total acid	Color change	Total acid	Color change
7 days	9.8	red ripe	13.2	ripe
14 days	8.1	dead ripe	10.1	red ripe
21 days	6.3	dead ripe	8.9	dead ripe
28 days	5.2	shriveling	6.6	some spoilage

TABLE 11. *Acidity changes in Globe tomatoes stored at 40°F., Fall crop 1930*

Length of storage period	Ripe				Green mature			
	Total acid	pH	Active acidity units	Color change	Total acid	pH	Active acidity units	Color change
7 days	10.1	4.33	433	red ripe	15.7	3.95	1125	yellowish green
14 days	9.7	4.40	400	red ripe	14.6	3.93	1175	pink showing at blossom end
21 days	9.3	4.38	420	lighter red	14.1	3.97	1075	pink showing at blossom end
28 days	8.1	4.45	357	lighter red	12.7	4.0	1000	pink showing at blossom end
35 days	6.9	4.45	357	lighter red	11.3	4.10	800	pink showing at blossom end
42 days	6.7	4.50	315	orange red	10.3	4.20	630	soft but no spoilage
49 days	5.4	4.50	315	orange red, watery				



TABLE 12. *Acidity changes in Globe tomatoes stored at 50°F., fall crop 1930*

Length of storage period	Ripe				Green mature			
	Total acid	pH	Active acidity units	Color change	Total acid	pH	Active acidity units	Color change
7 days	10.9	4.40	400	red ripe	16.5	3.90	1250	considerable coloring
14 days	9.9	4.38	420	red ripe	10.9	4.10	800	one-half ripe
21 days	8.8	4.40	400	red ripe	9.2	4.15	715	one-half ripe
28 days	6.5	4.45	357	red ripe	8.9	4.25	565	red ripe
35 days	6.5	4.50	315	red ripe	8.5	4.35	450	red ripe
42 days	6.5	4.50	315	few specimens	8.4	4.40	400	red ripe
49 days	5.8	4.60	250	break down	7.3	4.40	400	dead ripe

TABLE 13. *Acidity changes in Globe tomatoes stored at 70°F., fall crop 1930*

Length of storage period	Ripe					Green mature		
	Total acid	pH	Active acidity units	Color Change	Total acid	pH	Active acidity units	Color change
7 days	7.7	4.50	315	deep red	8.5	4.0	1000	nearly ripe
14 days	6.6	4.60	250	dead ripe	7.7	4.35	450	deep red
21 days	5.8	4.60	250	soft	6.4	4.5	315	dead ripe
28 days	5.4	4.70	200	some spoilage	6.4	4.5	315	slight shriveling
35 days	5.2	4.70	200	some spoilage	6.4	4.6	250	shriveled
42 days					6.1	4.63	215	badly shriveled

TABLE 14. *Acidity changes in Globe tomatoes, stored at 40°F., spring crop 1930*

Length of storage period	Ripe				Green mature			
	Total acid	pH	Active acidity units	Color change	Total acid	pH	Active acidity units	Color change
Initial acid	8.1	4.33	433	ripe red	15.2	4.0	1000	yellowish green
1 day	7.8	4.35	450	no change	15.0	3.95	1125	no change
2 days	7.7	4.40	400	no change	14.7	4.0	1000	no change
3 days	7.5	4.35	450	no change	14.8	4.0	1000	no change
4 days	7.8	4.38	420	no change	14.4	4.05	900	no change
5 days	7.9	4.40	400	no change	14.1	4.10	800	no change
6 days	7.7	4.40	400	no change	14.2	4.05	900	no change
7 days	7.8	4.45	357	no change	14.1	4.05	900	faint pink at blossom end

TABLE 15. *Acidity changes in Globe tomatoes stored at 50°F., spring crop 1930*

Length of storage period	Ripe				Green mature			
	Total acid	pH	Active acidity units	Color change	Total acid	pH	Active acidity units	Color change
Initial acid	8.1	4.33	433	red ripe	15.0	4.0	1000	yellowish green
1 day	7.9	4.35	450	no change	14.0	4.0	1000	yellowish green
2 days	7.5	4.40	400	no change	13.2	4.10	800	faint pink at blossom end
3 days	7.6	4.40	400	no change	11.4	4.15	715	faint pink at blossom end
4 days	7.3	4.40	400	no change	10.4	4.20	630	increase in color
5 days	7.0	4.45	357	no change	10.1	4.15	715	increase in color
6 days	7.1	4.40	400	no change	10.0	4.20	630	increase in color
7 days	6.9	4.45	357	no change	9.6	4.25	565	almost half ripe



TABLE 16. *Daily acidity changes in Globe tomatoes stored at 70°F., spring crop 1930*

Length of storage period	Ripe				Green mature			
	Total acid	pH	Active acidity units	Color change	Total acid	pH	Active acidity units	Color change
Initial acid	7.8	4.40	400	red ripe	15.0	4.0	1000	yellowish green
1 day	7.7	4.40	400	red ripe	13.2	4.10	800	yellowish green
2 days	7.7	4.40	400	red ripe	12.4	4.10	800	trace of pink at blossom end
3 days	6.1	4.50	315	red ripe	9.6	4.15	715	coloring rapidly
4 days	5.9	4.50	315	red ripe	9.4	4.30	500	one-half ripe
5 days	5.6	4.50	315	red ripe	9.2	4.40	400	
6 days	5.5	4.50	315	red ripe	8.6	4.45	357	
7 days	5.3	4.55	285	red ripe	8.5	4.45	357	ripe

TABLE 17. *Loss of moisture in storage*

Length of storage	Percentage		Percentage		Percentage	
	Ripe	40°F. Green mature	Ripe	50°F. Green mature	Ripe	70°F. Green mature
1 week	1.6	1.8	2.0	4.8	5.3	8.0
2 weeks	2.3	3.4	4.0	7.0	10.8	12.8
3 weeks	3.7	4.8	5.6	9.3	16.5	17.9
4 weeks	5.5	6.3	7.4	11.2	25.3	22.4
5 weeks	7.4	7.5	9.5	13.1		
6 weeks		9.5		15.1		

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# THE PRESENCE OF MYCELIUM AND OOSPORES OF CERTAIN DOWNY MILDEWS IN THE SEEDS OF THEIR HOSTS

I. E. MELHUS

*From the Department of Botany, Iowa State College*

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Little is known about the presence and the functioning of the mycelium and oospores of the Peronosporales in seeds of their hosts. Thaxter (8) suspected that *Phytophthora phaseoli* Thaxt. in the lima bean was seed borne, and Clinton (4) first found the oospores of this parasite in the seeds. However, when the seeds germinated the seedlings were not infected. Clinton suggested that primary infection of young seedlings originated from zoospores coming from the oospores carried over in the seed. Angell (1) believed that *Peronospora* sp. on tobacco could be transmitted by seed from badly infected fields. Recently, Cook (5) has reported the presence of mycelium of *Peronospora schleideni* Ung. in the pericarp and inner layers of the seed coat of *Allium cepa* L. and suggested the possibility of seed transmission. Leach (6) believes he has been able to secure infection on sugar and garden beet seedlings by planting seed matured on sugar beets infected with *Peronospora schachtii* Fekl. He also found mycelium and oospores in the coat of mature seed balls. There are many other species of Peronosporales that attack the floral parts of their hosts, but nothing is known about the relation of the parasite to the seed.

It is the purpose of this paper to record data showing the presence of mycelium and oospores in the seeds of plants attacked by three species of Peronosporales, *Peronospora alsinearum* Casp., *P. viciae* Berk., *P. effusa* (Grev.) Rabh., and *Cystopus bliti* (Biv.) de Bary

## PERONOSPORA ALSINEARUM CASP.

De Bary (2) noted that *Peronospora alsinearum* Casp. lived through the winter in the stems of *Stellaria media* (L.) Cyrill. In the spring new shoots were yellow and the parenchyma was filled with mycelium which fruited quickly. On examining the stems which had lived through the winter, the new mycelium could be distinguished from the old because the former had denser walls and fewer branches, but de Bary makes no reference to having observed the fungus on the seeds.

In the Spring of 1915, the writer collected *Peronospora alsinearum* on *Cerastium viscosum* L. on the banks of the Potomac River in Virginia, near the District of Columbia. The downy mildew was not only general on all the plants, but in a few the stems were hypertrophied and distorted, caused by the parasite. It was noted that the flowers and ripening fruit of the infected plants were attacked also. In some cases the *Peronospora* occurred on quite large ovaries where the seeds were approaching maturity.

Different lots of the infected ovaries were collected and placed in Merkel's and Fleming's killing solution. Later the ovaries were sectioned

and stained. Mycelium of *Peronospora alsinearum* was present in the walls of the ovary and seeds. Part of the pod as well as the central placenta showed numerous oospores and mycelium. Within the seeds and often enclosed by the cutinous epidermis and seed coat were many spores and mycelial threads, as shown in figure 1<sup>1</sup>. Some seeds were normal, while others in the same pod contained many oospores. In one case 13 oospores were observed in a single section. The badly infested seeds were smaller than normal and often distorted. In no case were spores or mycelium found within the cotyledons.

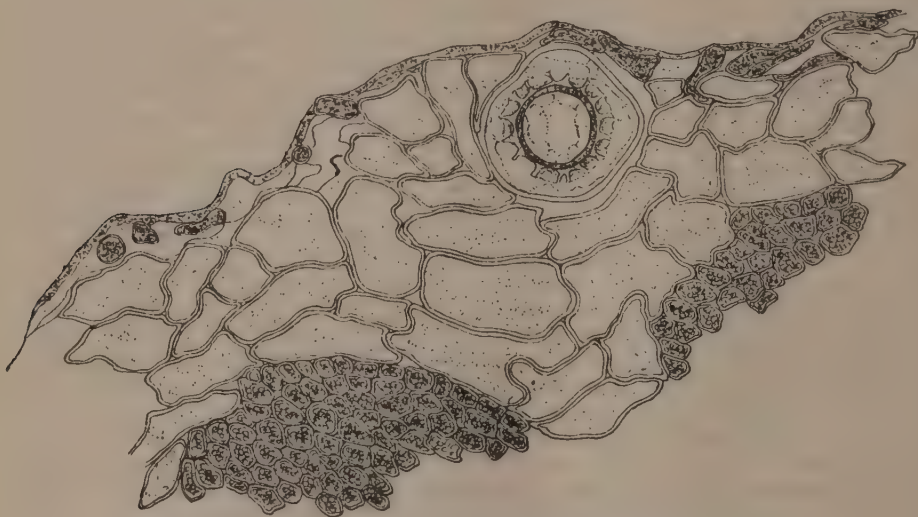


Fig. 1. Cross sections of ovaries of *Cerastium viscosum*, showing an oospore of *Peronospora alsinearum* embedded within tissue.

#### PERONOSPORA VICIAE BERK.

In 1912 the pea crop in Wisconsin was quite generally infected with downy mildew, *Peronospora viciae*. As described by Smith (7) and others, the fungus sporulates profusely on the lower leaves, forming a dull gray mat. The fungus not only attacks the leaves, but also the stems, flowers and pods. The stems are distorted, bright yellow in color, and may be filled with oospores. The mycelium becomes systemic in many of the plants. In such cases the whole aspect of the plant is changed. Its rich green color becomes a pale yellow, and there is a tendency of the plant to be more erect in habit. The mycelium attacks the developing pod and causes the walls of the ovaries to enlarge and become discolored, and a few of the pea seeds fail to develop. When such pods are opened there is no evidence of the parasite on the seed or the inner surfaces of the ovary walls. Some of the seeds may appear quite normal in color, size, and shape.

<sup>1</sup>The author is indebted to Dr. L. W. Durrell for the drawings used in figures 1 and 2.



In order to determine whether the mycelium of *Peronospora viciae* was present in the seeds, some were removed from infected pots and placed in Fleming's killing solution, sectioned, and stained. Mycelium was found commonly in such seeds in the intercellular spaces of the seed coat as shown in figure 2.



Fig. 2. Section of seed of *Pisum sativum* showing mycelium of *Peronospora viciae* in tissues.

#### CYSTOPUS BLITI (BIV.) de BARY

In the Spring and Fall of 1920, *Cystopus bliti* was unusually common on *Amaranthus retroflexus* L. at Ames, Iowa. It appeared in localized spots on this host early in the spring as soon as the plants became two or three inches high. At this stage of growth of the host, the parasite is never systemic. The amount of infection increases as the host develops until finally the leaves are infected generally. Following conidial fructification, oospores are formed in the tissues of the leaves in such quantities that they can readily be seen with a hand lens and when the oospores are ripe and black they are even perceptible by the naked eye. The greatest number of oospores are produced in the infected stems and flowers. Early stages of infection cause the flowers to change from a green to reddish or light brown color as the seeds set and mature. The stems and flowers become hypertrophied and distorted. These symptoms are so positive and the color so striking that one can often detect infected racemes at a considerable distance.

Ripening seeds from such infected spikes were collected and prepared for sectioning. The mycelium and mature oospores of *Cystopus bliti* were found in the floral bracts, ovary walls, and inside the seed coat in close proximity to the tips of the embryonic cotyledons.

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## 2-METHYL-3-FUROIC ACID

HENRY GILMAN AND ROBERT R. BURTNER

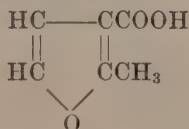
*From the Chemical Laboratory of Iowa State College*

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### INTRODUCTION

In continuation of studies concerned with orientation in the furan nucleus<sup>1</sup>, it was necessary to extend the present very limited number of methods available for introducing substituents in the 3- and in the 4- positions of the furan cycle. Advances in determining the mode of substitution and the positions assumed by entering groups, both in the relative and the absolute methods, are dependent on the introduction of substituents which lend themselves to removal or replacement. Among such groups is the carboxyl which can generally be removed under moderate conditions by simple pyrolysis.

One of these important key-compounds is 2-methyl-3-furoic acid.



This compound can be prepared by a method developed by Benary<sup>2</sup>. The synthesis involves the interaction of  $\alpha$ ,  $\beta$ -dichloroethyl ether, ethyl acetate and aqueous ammonia, and the products are 2-methyl-pyrrole-3-carboxylic acid (or its ethyl ester) and 2-methyl-3-furoic acid. The yields are distressingly low, and the disadvantage of the original synthesis is accentuated by the necessity for removing the pyrrole ester. It has been shown, in the present study, that these difficulties can be circumvented, in large part, by the use of sodium hydroxide as the condensing agent. With this simple modification it is possible to prepare 2-methyl-3-furoic acid conveniently and in satisfactory yields.

The same procedure is admirably suited for the synthesis of other 3-carboxylated furans, and it has been applied by us to the preparation of 2-furyl-3-furoic acid<sup>3</sup>, 2, 4-dimethyl-3-furoic acid<sup>4</sup>, and 2-phenyl-3-furoic acid<sup>5</sup>. Further details of these compounds will be published later, but it can be stated now that the yields of these three acids were not so high as those obtained with 2-methyl-3-furoic acid. The 2-furyl- and 2-phenyl-3-

<sup>1</sup>Gilman and Wright, *Iowa State College Jour. Sci.*, **5**:85 (1931).

<sup>2</sup>Benary, *Ber.*, **44**:493 (1911). See, also, Plancher and Albini, *Atti. acad. Lincei* [5] **13**, I, 39 (1904).

<sup>3</sup>Kondo and Suzuki, *J. Pharm. Soc. Japan*, **554**:501 (1927) [*C. A.*, **21**:3362 (1927)].

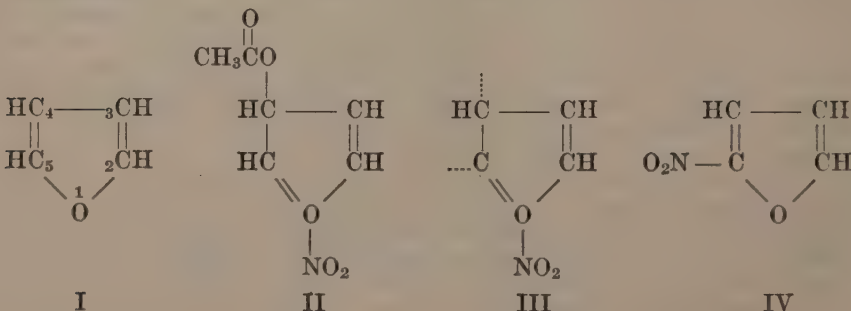
<sup>4</sup>Plancher and Albini, *Atti. accad. Lincei* [5] **13**, I, 39 (1904).

<sup>5</sup>Fujita, *J. Pharm. Soc. Japan*, **519**:450 (1925) [*C. A.*, **20**:757 (1926)].

furoic acids are admirably suited as starting materials for the preparation of compounds which should serve to throw light on the stereo or spatial configuration of furan compounds after the related diphenyl type of isomerism. On the basis of this well-established type of stereoisomerism which found its fullest confirmation in the resolution of racemates, it appears altogether reasonable to expect that related optically active furan compounds will be obtained with the introduction of proper substituents in the critical positions.

Very closely related to the problem of di-aryl isomerism is the reaction between furfuryl chloride ( $C_4H_3OCH_2Cl$ ) and magnesium in ether<sup>6</sup>. The furfuryl chloride, which is analogous to benzyl chloride, reacts with the activated magnesium-copper alloy in ether to give as one of the products *sym.*-di-2-furylethane. The identity of this compound was established by comparison with the known difurylethane, prepared by the reduction of *sym.*-di-2-furylethylene<sup>7</sup>. Both the known ethane and that synthesized from furfuryl chloride and magnesium gave the same nitro compound (melting at  $158^\circ$ ), as determined by the method of mixed melting points. The formation of *sym.*-di-2-furylethane is unequivocal evidence for the formation of furfurylmagnesium chloride, because it has been demonstrated that free radicals (in this case, the furfuryl radical) are precursors of organomagnesium compounds. The organomagnesium compound and its reactions will be reported subsequently.

The ethyl ester of 2-methyl-3-furoic acid was nitrated, and the nitro compound so formed probably has the nitro group in the 5-position, in accordance with the general tendency of substituents to occupy the 2- and 5-positions. We have hitherto<sup>1,8</sup> assumed that substitution to these positions occurred by virtue of a 1, 4-addition to a conjugated system, the key-elements of which are carbon atoms. As mentioned earlier<sup>8</sup>, there are several possible conjugated systems in furan (I) and its non-hydrogenated derivatives.



One of these, which turns on en-onium conjugation, is the system 1, 5, 4 in which it is assumed that the oxygen atom (element 1 in the cycle) is un-

<sup>6</sup>These reactions were carried out by Mr. G. F. Wright.

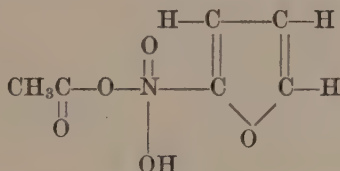
<sup>7</sup>The reduction was effected by Mr. W. C. Ashley in accordance with the procedure of Reichstein, *Helvetica Chim. Acta* 13:348 (1930), whose structure for difurylethane is assumed until experiments now in progress definitely establish its constitution.

<sup>8</sup>Gilman and Wright, *J. Am. Chem. Soc.*, 52:3349 (1930). See, Note 15, on p. 3351.



saturated and so conjugated with the ethylenic linkage between carbon atoms 5 and 4. Assuming that the effective nitrating agent is acetyl nitrate, for which there is reasonable support, then nitration of furan (or its derivatives) would give, on this basis, Compound II. By loss of  $\text{CH}_3\text{COO}$  and H (generally by means of pyridine) Compound III would form. A re-distribution of valences in III would give rise to Compound IV. An interpretation of this type makes unnecessary any explanation postulated on ring splitting<sup>9</sup>. A study of the intermediate compounds by chemical reactions (addition, ozonization, etc.) and by absorption spectra<sup>10</sup> should help to support or disprove these postulates.

Because of the relatively saturated nature of the intermediate, it is quite reasonable to postulate that the ring is neither split, nor altered in its so-called aromatic unsaturation. One way of expressing this is the following compound formed from either acetyl or diacetyl nitric or ortho-nitric acids.



This compound would then readily lose acetic acid to give the nitrofuran. It has since been determined that the acetic acid is removed not only by basic nitrogen compounds, but also by mild inorganic bases like barium hydroxide, and agencies like moderate heat.

Incidentally, it is interesting to reflect that only the position of the methyl group is established in 2-methyl-3-furoic acid, and this was done by decarboxylation to give the known sylvan<sup>2</sup>.

#### EXPERIMENTAL PART

*Preparation of 2-Methyl-3-Furoic Acid.* To a mixture of 26 g. (0.2 mole) of ethyl acetoacetate and 40 g. (0.3 mole) of  $\alpha$ ,  $\beta$ -dichloroethyl ether, 240 cc. of 10 per cent aqueous sodium hydroxide were added slowly with vigorous stirring. A considerable amount of heat is evolved and the reaction is complete in about five minutes. The reaction mixture is then cooled and extracted with ether, dried over anhydrous sodium sulfate and the ether removed by distillation. The residue is fractionated under reduced pressure and the fraction boiling between  $70^\circ$ - $100^\circ/25$  mm. is collected. This product may be purified by subsequent distillation collecting the fraction at  $85$ - $87^\circ/20$  mm., but it was found that this operation was unnecessary. The ester thus formed is saponified by refluxing with 0.2 mole of alcoholic potassium hydroxide for two hours, at the end of which

<sup>9</sup>It must, however, be remembered that in some nitrations like that of furylacrylic acid, there is no apparent addition of the groups which go to make up  $\text{CH}_2\text{COOH}$ . In these cases the explanation probably lies in part in the new conjugated systems which are set up outside the cycle.

<sup>10</sup>Hughes and Johnson, *J. Am. Chem. Soc.*, **53**:737 (1931).

period the material is distilled to dryness under reduced pressure, the source of heat being an oil bath. The residue is cooled and dissolved in about 100 cc. of water, placed in an ice bath and carefully acidified with dilute hydrochloric acid. A yellow precipitate forms which is filtered and recrystallized from hot water as pale yellow or white needles melting at 101°. Further amounts may be recovered by acidifying the aqueous layer from the original ethereal extraction. The yield is 6.8 g. or 26.9 per cent of the theoretical based on acetoacetic ester.

*Nitration of Ethyl 2-Methyl-3-Furoate.* 24 g. (0.15 mole) of ethyl 2-methyl-3-furoate dissolved in 30.6 g. of acetic anhydride were added dropwise with stirring at  $-25^{\circ}$  to the nitrating mixture prepared by the dropwise addition of 52.5 g. (0.83 mole) of fuming nitric acid to 84 g. of acetic anhydride at  $0^{\circ}$ . Subsequent to the addition of the ester to the nitrating mixture, the reactants were stirred for an additional thirty minutes and then poured onto approximately 500 g. of cracked ice. The mixture was partially neutralized with 50 per cent sodium hydroxide at which point an oily layer separated to the bottom. Then 150 cc. of ether were added and the neutralization completed with a saturated solution of sodium bicarbonate, the ether being added to prevent violent ebullition. The reaction mixture was then extracted with ether, and a volume of pyridine equal to the amount of oil was added with stirring. It was then allowed to stand for twenty-four hours and the ether distilled off. The major portion of the pyridine was distilled off under reduced pressure and the residue was chilled and acidified with 10 per cent hydrochloric acid whereupon a mass of reddish brown crystals formed. The crystalline material was filtered by suction and washed with water. The crude ethyl 5-nitro-2-methyl-3-furoate melted at  $51^{\circ}$ . When recrystallized from ether, it forms pale yellow needles melting at  $52.5^{\circ}$ . The yield was 8.5 g. or 28.3 per cent of the theoretical amount.

*Analysis:* Calcd. for  $C_8H_9O_5N$ : C, 48.24; H, 4.52. Found: C, 48.45; H, 4.38.

#### SUMMARY

An improved method has been described for the preparation of 2-methyl-3-furoic acid, an important starting compound in orientation studies. Related types have also been prepared by the same procedure, and these compounds are being investigated in a study of stereoisomerism in the furan series.

# THE REDUCTION OF HYDROFURAMIDE TO TRI-*a*-FURFURYLAMINE

HENRY GILMAN AND J. B. DICKEY

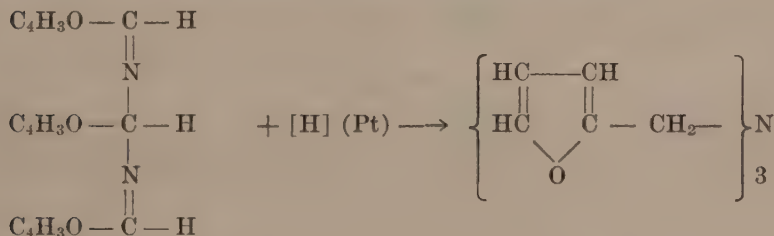
*From the Chemical Laboratory of Iowa State College*

Accepted for publication March 10, 1931

## INTRODUCTION

The utilization of furfural and of furan types in general is markedly circumscribed by the uncommon difficulty of preparing amino compounds, either inexpensively or in some cases<sup>2</sup> without regard to the cost involved. We are now describing a simple method for the preparation of tri-*a*-furfurylamine in satisfactory yields and almost directly from the very low-priced furfural.

First, hydrofurfuramide is prepared in excellent yields by the ready condensation of furfural with ammonia. Second, the hydrofurfuramide is reduced catalytically in satisfactory yields to tri-*a*-furfurylamine.



The tri-*a* furfurylamine so obtained is relatively stable, and is being tested for a miscellany of purposes. The results of these tests as well as a study of readily accessible derivatives<sup>3</sup> from this tertiary amine will be reported later. Incidentally, the method of reduction should make available substituted tri-*a*-furfurylamines, inasmuch as an increasing number of substituted furfurals are being prepared<sup>4</sup>.

<sup>1</sup>This is one of a series of studies in organic chemistry concerned with the utilization of agricultural wastes. The authors gratefully acknowledge help from the Industrial Science Research Fund for the partial defrayal of expenses incurred in this investigation.

<sup>2</sup>Gilman and Wright, *J. Am. Chem. Soc.*, **52**, 2550 (1930).

<sup>3</sup>Zanetti and Beckmann, *ibid.*, **50**, 2031 (1928) prepared the three furfurylamines from furfuryl bromide and ammonia and characterized them as their hydrochlorides, chloroplatinates and chloroaurates.

<sup>4</sup>For earlier reports on such studies from this Laboratory see articles in *Iowa State College Jour. Sci.*, *J. Am. Chem. Soc.*, and *Ecc. trav. chim.*

## EXPERIMENTAL PART

In a typical reduction experiment, 0.3 g. of Adams' platinum oxide catalyst was added to 55 g. (0.205 mole) of hydrofuramide in 150 cc. of glacial acetic acid and 25 cc. of 95 per cent ethyl alcohol. The mixture was shaken with hydrogen and 0.615 mole of hydrogen was added in four hours, after two reactivations. Subsequent to filtration of the black solution to remove the catalyst, the filtrate was neutralized with dilute sodium carbonate and extracted with ether. The ether solution was washed with water, dried over sodium sulfate and distilled. The yield of tri- $\alpha$ -furfurylamine boiling at 133-138°/1 mm. was 21 g. or 38.2 per cent. The amine is a yellow oil of slight odor and of relative high stability for a furyl amine. Very probably subsequent experiments, particularly on a larger scale and with other reductants, will show that the yield can be improved. The yield reported has been checked by more than one person.

The hydrochloride was prepared in accordance with the directions of Zanetti and Beckmann<sup>3</sup> and agreed in melting point with their salt. Also, there was no depression in a mixed-melting point determination with an authentic specimen of the hydrochloride as prepared from furfuryl bromide and ammonia<sup>3</sup>. The salt is also quite stable even in the atmosphere.

The picrate of tri- $\alpha$ -furfurylamine was prepared by treating a dilute solution of the amine in ether with a concentrated solution of excess picric acid in ether. A heavy yellow crystalline solid was obtained in this way, and on crystallization from 95 per cent ethyl alcohol it melted at 132°.

The authors are grateful to W. H. Zuschwerdt for assistance.

## SUMMARY

The catalytic reduction of hydrofuramide, which is obtainable directly in excellent yields from furfural and ammonia, gives tri- $\alpha$ -furfurylamine. This tertiary amine is probably the most accessible and least expensive furyl amine.



# FURTHER STUDIES ON THE INSECT FAUNA OF IOWA PRAIRIES

GEORGE O. HENDRICKSON

*From the Department of Zoology and Entomology, Iowa State College*

Accepted for publication April 28, 1931

During the past six years the author has made studies of the species of insects at several communities of prairie plants in forty locations in Iowa. These plant communities were classified in accordance with a plan outlined by Clements (1920). A list of these locations, plant communities and 1,175 species and subspecies of associated insects has been published previously (Hendrickson, 1930).

This article includes a report on 144 species more recently identified and represents an addition to the aforementioned list.

## ANNOTATED LIST

### ORDER HOMOPTERA

The species were identified by Dr. D. M. DeLong.

#### *Draeculacephala minor* Walk.

At *Stipa spartea*—*Andropogon scoparius* association, 2 miles north of Ames, May 15, 1926, one specimen.

#### *Deltocephalus sylvestris* O. & B.

At *Stipa spartea*—*Andropogon scoparius* association, 2 miles north of Ames, May 7, 1926, one specimen.

#### *Dikraneura abnormis* Walsh

At *Andropogon furcatus* consocieties, 2.5 miles south of Ames, May 17, 1926, one specimen.

### ORDER COLEOPTERA

The species were identified by Dr. G. M. Stirrett, and Messrs. Chas. Schaeffer and W. J. Brown.

#### *Choleva terminans* Lec.

At *Bouteloua hirsuta*—*B. curtipendula* association, 5 miles south of Stanhope, Oct. 20, 1928, one specimen.

#### *Oxytelus suspectus* Csy.

At *Andropogon furcatus* consocieties, 2.5 miles south of Ames, June 5, 1927, one specimen. Swept from flowers of *Erigeron ramosus*, 1 mile south of Amana, June 23, 1928, one specimen.

#### *Platystethus americanus* Er.

At *Andropogon furcatus* consocieties, 2.5 miles south of Ames, Mar. 25, 1928, one specimen.

*Stenus egenus* Er.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, common. Earliest adult, April 22, 1928; latest adult, Sept. 7, 1928.

*Stenus punctatus* Er.

At *Spartina consocias*, common. Earliest adult, Apr. 12, 1926; latest adult, May 23, 1928.

*Paederus littorarius* Grav.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, 2.5 miles south of Ames, Apr. 16, 1927, and 8 miles southeast of Britt, May 19, 1928, three specimens.

*Pseudolathra analis* Lec.

Under a stone, *Stipa spartea*—*Andropogon scoparius* association, 2 miles north of Ames, one specimen.

*Philonthus quisquiliaris* Gyll.

At *Spartina consocias*, 8 miles southeast of Britt, May 19, 1928, two specimens.

*Philonthus microphthalmus* Horn

At *Spartina consocias*, 8 miles southeast of Britt, May 19, 1928, one specimen.

*Staphylinus cinnamopterus* Grav.

Under a stone, at *Stipa spartea*—*Andropogon scoparius* association, 2 miles north of Ames, Apr. 25, 1927, one specimen.

*Tachyporus chrysomelinus* Linn.

At *Andropogon furcatus* consocias, common. Earliest adult, Apr. 5, 1928; latest adult, Sept. 15, 1928.

*Tachyporus nitidulus* Fab.

At *Andropogon furcatus* consocias, 5 miles east of Renwick, May 19, 1928, one specimen.

*Mycetoporus humidus* Say

At *Andropogon furcatus* consocias, 5 miles east of Renwick, May 19, 1928, one specimen.

*Mycetoporus lucidulus* Lec.

At *Andropogon furcatus* consocias, 5 miles east of Renwick, May 19, 1928, two specimens, and 2.5 miles south of Ames, Apr. 12, 1928, one specimen.

*Tinotus paratus* Csy.

At *Stipa spartea*—*Andropogon scoparius* association, 5 miles south of Stanhope, Aug. 19, 1927, one specimen.

*Atheta nigrītula* Grav.

At *Andropogon furcatus* consociēs, common. Earliest adult, Apr. 25, 1928; latest adult, Oct. 20, 1928.

*Atheta palustris* Kiesw.

At *Stipa spartea*—*Andropogon scoparius* association, 5 miles south of Stanhope, Oct. 20, 1928, one specimen.

*Datomicra zosteræ* Thoms.

At *Andropogon furcatus* consociēs, 8 miles southeast of Britt, May 19, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 miles south of Stanhope, Oct. 20, 1928, one specimen. At *Spartina* consociēs, 2.5 miles south of Ames, June 5, 1927, one specimen.

*Colpodota aterrima* Grav.

At *Andropogon furcatus* consociēs, 2.5 miles south of Ames, Apr. 22, 1928, one specimen.

*Coprothassa sordida* Marsh.

At *Stipa spartea*—*Andropogon scoparius* association, 5 miles south of Stanhope, Oct. 20, 1928, one specimen.

*Baryodma verna* Say

At *Stipa spartea*—*Andropogon scoparius* association, 5 miles south of Stanhope, Oct. 20, 1928, one specimen.

*Anthicus ephippium* Laf.

At *Andropogon furcatus*—*Spartina Michauxiana* associēs, May 9, 19, 1928, common.

*Melanophthalma distinguenda* Com.

At *Andropogon furcatus*—*Spartina Michauxiana* associēs, common. Earliest adult, Mar. 25, 1928; latest adult, Oct. 20, 1928.

*Paratenetus fuscus* Lec.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1928, one specimen.

*Pachybrachys calcaratus* Fall.

At *Andropogon furcatus* consociēs, 2.5 miles south of Ames, June 5, 1927, one specimen.

*Disonycha pennsylvanica* Ill.

At *Polygonum amphibium* sociēs, common. Earliest adult, May 12, 1926; latest adult, Sept. 16, 1928.

*Disonycha quinquevittata* Say

At *Spartina* consociēs, 1 mile south of Amana, Aug. 12, 1927, two specimens.

*Disonycha latifrons* Schaeef.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, July 25, 26, 1928, numerous.

*Luperaltica fuscula* Lec.

Swept from flowers of *Andropogon furcatus*, 1 mile south of Amana, Aug. 25, 1928, one specimen, and from flowers of *Solidago rigida*, 5 miles south of Stanhope, Sept. 19, 1928.

*Chaetocnema confinis* Cr.

Swept from flowers of *Solidago canadensis*, *Gaura* sp., *Cassia Chamaecrista* and *Andropogon furcatus*, 1 mile south of Amana, Aug. 12, 25, 1927. Common.

*Longitarsus turbatus* Horn

At *Andropogon furcatus* consocieties, 2.5 miles south of Ames, Mar. 25, 1928, one specimen.

*Longitarsus melanurus* Melsh.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, numerous. Earliest adult, Mar. 25, 1928; latest adult, June 23, 1928.

## ORDER DIPTERA

The species were identified by Drs. J. M. Aldrich, C. H. Curran, and Chas. T. Greene.

*Chaoborus punctipennis* Say

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 3 miles south of Muscatine, Sept. 1, 1928, two specimens.

*Aldrovandiella halterata* Meig.

At all plant communities. Numerous at *Spartina* consocieties, Lake Amana, June 23, 1928.

*Diaphorus opacus* Loew

At *Spartina* consocieties, Lake Amana, June 23, 1928, one specimen.

*Sympycnus nodatus* Loew

At *Andropogon furcatus*—*Spartina Michauxiana* associates, common. Earliest adult, May 12, 1927; latest adult, June 23, 1928.

*Dolichopus cuprinus* Wied.

At *Carex* societies, 7.75 miles northwest of Thompson, June 30, 1928, one specimen. At *Andropogon furcatus* consocieties, 4 miles north of LeMars, July 26, 1928, one specimen.

*Dolichopus renidescens* Mel. & Brues

At *Spartina* consocieties, common. Earliest adult, May 2, 1928; latest adult, Sept. 19, 1928.



*Gymnopternus barbatulus* Loew

At *Spartina* consociies, Lake Amana, June 23, 1928, one specimen.

*Platypalpus tersus* Coq.

Swept from flowers of *Anemone canadensis*, 1 mile south of Amana, June 23, 1928, one specimen.

*Tachydromia maculipennis*

At *Stipa spartea*—*Andropogon scoparius* association, common. Earliest adult May 9, 1928; latest adult, June 15, 1928.

*Lonchoptera furcata* Fall

At *Spartina* consociies, common. Earliest adult, Mar. 25, 1928; latest adult, May 20, 1927.

*Aphiochaeta iroquoiana* Mall.

At *Spartina* consociies, 2.5 miles south of Ames, July 29, 1928, one specimen.

*Aphiochaeta minor* Zett.

At *Spartina* consociies, 2.5 miles south of Ames, July 29, 1928, one specimen.

*Aphiochaeta perplexa* Mall.

At *Stipa spartea*—*Andropogon scoparius* association, 5 miles south of Stanhope, Oct. 20, 1928, one specimen.

*Paragus angustifrons* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, June 30, 1928, one specimen.

*Calythea albicincta* Fall.

At *Stipa spartea*—*Andropogon scoparius* association, Ocheyedan Mound, July 23, 1928, one specimen.

*Hylemyia cinerella* Fall.

At *Andropogon furcatus* consociies, 7.75 miles northwest of Thompson, June 30, 1928, one specimen.

*Coenosia humilis* Meig.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, common. Earliest adult, May 9, 1928; latest adult, May 20, 1928.

*Schoenomyza chrysotoma* Loew

At *Andropogon furcatus*—*Spartina Michauxiana* associates, common. Earliest adult, Mar. 25, 1928; latest adult, May 20, 1928.

*Pseudoleria pectinata* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 5 miles south of Stanhope, Aug. 5, 1927, one specimen, and 2 miles north of Ames, June 19, 1926, one specimen.

*Leptocera limosa* Mg.

At *Spartina* consocieties, 2.5 miles north of Ames, May 7, 1928, one specimen.

*Tetanocera triangularis* Loew

At *Polygonum amphibium* societies, 3.5 miles north of Ledyard, July 7, 1928, two specimens.

*Tetanocera vicina* Macq.

At *Spartina* consocieties, Lake Amana, June 23, 1928, one specimen, and 10 miles southwest of Kelso, July 30, 1928, one specimen.

*Sepedon pusillus* Loew

At *Spartina* consocieties, 8 miles southeast of Britt, May 19, 1928, one specimen.

*Eumetopiella rufipes* Macq.

At *Polygonum amphibium* societies, .5 mile south of Missouri Valley, Aug. 1, 1928, one specimen.

*Dichaeta caudata* Fall

At *Spartina* consocieties, 8 miles southeast of Britt, May 19, 1928, one specimen.

*Psilopa atrimanus* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, May 18, 1928, one specimen, and 5 miles south of Stanhope, Oct. 20, 1928, one specimen.

*Philyria fuscicornis* Loew

At *Stipa spartea*—*Andropogon scoparius* association, numerous. Earliest adult, Apr. 16, 1928; latest adult, Apr. 25, 1927. At *Andropogon furcatus* consocieties, 2.5 miles south of Ames, Apr. 16, 1927, Mar. 25, 1928, four specimens.

*Parydra paullula* Loew

At *Spartina* consocieties, 8 miles southeast of Britt, May 19, 1928, one specimen.

*Scatella lugens* Loew

At *Spartina* consocieties, numerous. Earliest adult, Apr. 22, 1928; latest adult, May 20, 1927.

*Diplotoxa alternata* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, June 30, 1928, two specimens.

*Epichlorops exilis* Coq.

At *Carex* societies, 10 miles southwest of Kelso, July 30, 1928, one specimen.

*Hippelates flavipes* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, Aug. 6, 1928, one specimen.

*Crassiseta longula* Loew

At *Carex* societas, 7.75 miles northwest of Thompson, June 30, 1928, three specimens. At *Stipa spartea*—*Andropogon scoparius* association, 5 miles northwest of Buffalo Center, July 7, 1928, one specimen.

*Elachiptera costata* Loew

At *Spartina* consocietas, Lake Amana, June 23, 1928, one specimen, and 2.5 miles south of Ames, May 12, 1927, one specimen.

*Elachiptera longula* Loew

At *Spartina* consocietas, Lake Amana, June 23, 1928, two specimens, and 7.75 miles northwest of Thompson, June 30, 1928, one specimen.

*Siphonella parva* Adams

At *Stipa spartea*—*Andropogon scoparius* association, 5 miles south of Stanhope, June 15, 1928, one specimen.

*Oscinella ainsliei* Curran

At *Spartina* consocietas, 1 mile south of Amana, Aug. 12, 1927, and June 23, 1928; two specimens.

*Cerodontha dorsalis* Loew

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, Sept. 15, 1928, one specimen.

*Agromyza affinis* Mall.

At *Stipa spartea*—*Andropogon scoparius* association, Ocheyedan Mound, July 23, 1928, one specimen.

*Agromyza virens* Loew

At *Polygonum amphibian* societas, 2.5 miles south of Ames, Aug. 4, 1927, one specimen.

*Desmometopa sordidum* Fall.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 1 mile north of Reels City, Aug. 1, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 miles northwest of Buffalo Center, July 7, 1928, one specimen.

*Ochthiphila aridella* Fall.

At *Andropogon furcatus* consocietas, 5 miles east of Renwick, May 19, 1928, two specimens.

*Ochthiphila juncorum* Fall.

At *Spartina* consocietas, 5 miles east of Renwick, May 19, 1928, one specimen, and 4 miles northwest of Thompson, May 18, 1928, one specimen.

*Ockthipila polystigma* Meig.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, Aug. 6, 1928, two specimens, and 2 miles north of Ames, Sept. 1, 1926, one specimen.

*Hesperophasia setosa* Tns.

At *Stipa spartea*—*Andropogon scoparius* association, 2.5 miles north of Ames, July 8, 1925, one specimen. At *Carex* socius, 5 miles northwest of Buffalo Center, July 7, 1928, one specimen.

*Blepharigena spinosula* Big.

At *Stipa spartea*—*Andropogon scoparius* association, 5 miles south of Stanhope, Sept. 19, 1928, one specimen.

*Zenillia caesar* Ald.

At *Stipa spartea*—*Andropogon scoparius* association, 2 miles north of Ames, July 7, 1926, one specimen. At *Andropogon furcatus* consocius, 5 miles northwest of Buffalo Center, Sept. 16, 1928, one specimen.

*Alophora semicinerea* Meig.

At *Stipa spartea*—*Andropogon scoparius* association, 2 miles north of Ames, Sept. 24, 1926, two specimens.

*Clausicella usitata* Coq.

At *Stipa spartea*—*Andropogon scoparius* association, Aug. 7, 25, 1926, two specimens.

*Exorista mella* Walk.

Swept from flowers of *Solidago* sp., 2 miles north of Ames, Sept. 24, 1926, one specimen.

## ORDER HYMENOPTERA

The species were identified by Mr. R. A. Cushman, except *Ephedrus californicus* Baker, which was identified by Mr. A. B. Gahan.

*Cardiochiles seminiger* Cress.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 16, 1928, one specimen.

*Apanteles femur-nigrum* Prov.

At all major communities. Common at *Stipa spartea*—*Andropogon scoparius* association. Earliest adult, May 19, 1928; latest adult, Aug. 6, 1928.

*Apanteles* sp. near *femur-rubrum* Prov.

At *Spartina* consocius, Lake Amana, June 23, 1928, one specimen. Swept from flowers of *Anemone canadensis*, 1 mile south of Amana, June 23, 1928, and from flowers of *Cicuta maculata*, 5 miles northwest of Buffalo Center, July 7, 1928.



*Apanteles forbesi* Vier.

Swept from flowers of *Rudbeckia hirta*, 1 mile south of Amana, June 23, 1928, one specimen. At *Spartina* consocieties, 5 miles northwest of Buffalo Center, Sept. 16, 1928, one specimen.

*Apanteles lunatus* Pack.

Reared from a slender, pale green larva (*Geometridae*) that fed on flower of *Rudbeckia hirta*. The parasite spun its cocoon outside of the host, July 19, 1928, and the adult appeared July 28, 1928.

*Apanteles xylinus* Say

At *Andropogon furcatus* consocieties, 2.5 miles south of Ames, May 12, 1927, one specimen.

*Apanteles aristoteliae* Vier.

At *Stipa spartea*—*Andropogon scoparius* association, 6 miles northwest of Ledyard, Aug. 7, 1928, one specimen.

*Microgaster brittoni* Vier.

At *Spartina* consocieties, 1 mile south of Amana, Aug. 13, 1927, three specimens.

*Microgaster carinatus* Pack.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, June 23, 1928, one specimen.

*Microplitis melianae* Vier.

At all major communities except *Andropogon scoparius*—*Bouteloua curtipendula* association. Earliest adult, May 8, 1928; latest adult, Sept. 1, 1928.

*Microbracon americanus* Ashm.

At *Spartina* consocieties, 4 miles northwest of Thompson, May 18, 1928, one specimen.

*Microbracon caulicola* Gahan

At *Stipa spartea*—*Andropogon scoparius* association, 5 miles south of Stanhope, Aug. 9, 1928, one specimen. At *Spartina* consocieties, 10 miles southwest of Kelso, July 30, 1928, one specimen.

*Microbracon meromyzae* Gahan

At *Spartina* consocieties, 8 miles southeast of Britt, May 19, 1928, one specimen.

*Microbracon nuperus* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, Aug. 6, 1928, one specimen.

*Microbracon pygmaeus* Prov.

At *Andropogon furcatus* consocieties, 8 miles southeast of Britt, Aug. 9, 1928, one specimen. At *Stipa spartea*—*Andropogon scoparius* association, 5 miles south of Stanhope, Oct. 20, 1928, one specimen.

*Coelinidea ferruginea* Gahan

At *Spartina* consocies, Lake Amana, June 23, 1928, one specimen.

*Coelinidea meromyzae* Forbes

At *Spartina* consocies, Lake Amana, Aug. 31, 1927, one specimen.

*Banchus inermis* Prov.

At *Carex* sociies, 5 miles northwest of Buffalo Center, July 7, 1928, one specimen.

*Meteorus hyphanthiae* Riley

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, Sept. 15, 1928, one specimen.

*Meteorus politus* Prov.

At *Stipa spartea*—*Andropogon scoparius* association, 5 miles northwest of Buffalo Center, Aug. 6, 1928, one specimen. At *Andropogon furcatus* consocies, 5 miles east of Renwick, Aug. 9, 1928, one specimen.

*Euphorus mellipes* Cress.

Swept from flowers of *Erigeron ramosus*, 1 mile south of Amana, June 23, 1928, two specimens.

*Bracon vulgaris* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, June 30, 1928, three specimens.

*Euphoriana uniformis* Gahan

At *Spartina* consocies, 1 mile south of Amana, Aug. 13, 1928, one specimen. At *Andropogon furcatus* consocies, 2.5 miles south of Ames, July 11, 1928, one specimen, and 5 miles east of Renwick, Aug. 9, 1928, one specimen.

*Bassus agilis* Cress.

At *Polygonum amphibium* sociies, 2.5 miles south of Ames, Aug. 4, 1927, one specimen.

*Bassus buttricki* Vier.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, one specimen.

*Bassus discolor*, Cress.

At *Spartina* consocies, Lake Amana, Aug. 31, 1927, two specimens.

*Bassus perforator* Prov.

At *Andropogon furcatus*—*Sorghastrum nutans* associies, 6 miles south of Washington, Sept. 7, 1927, one specimen.

*Bassus terminatus* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, June 30, 1928, one specimen.

*Bassus tibiator* Prov.

At *Bouteloua hirsuta*—*B. curtispindula* association, 5 miles south of Stanhope, Sept. 19, 1928, one specimen.

*Ascogaster mimeticus* Vier.

At *Carex socias*, 1 mile south of Amana, June 23, 1928, one specimen.  
At *Andropogon furcatus*—*Sorghastrum nutans* associates, 6 miles south of Washington, Sept. 7, 1927, three specimens.

*Urosigalphus femoratus* Cwfd.

Swept from *Helianthus grosseserratus*, 1 mile south of Amana, Aug. 13, 1927, two specimens.

*Rogas terminalis* Cress.

At *Spartina consocias*, common. Earliest adult, Aug. 13, 1927; latest adult, Sept. 16, 1928.

*Schizoprymnus texanus* Cress.

At *Andropogon furcatus* consocias, 6 miles northwest of Ledyard, July 7, 1928, one specimen.

*Yelicones delicatus* Cress.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 3 miles south of Muscatine, Sept. 1, 1928, one specimen.

*Ephedrus californicus* Baker

Swept from flowers of *Anemone canadensis*, 1 mile south of Amana, June 23, 1928, one specimen.

*Cymodusa distincta* Cress.

At *Andropogon furcatus* consocias, 2 miles north of Ames, Aug. 26, 1926, one specimen, and 5 miles east of Renwick, May 19, 1928, one specimen.

*Sagaritis consimilis* Ashm.

At *Stipa spartea*—*Andropogon scoparius* association, 6 miles northwest of Ledyard, July 7, 1928, one specimen. At *Andropogon furcatus* consocias, common. Earliest adult, May 19, 1928; latest adult, Aug. 4, 1928.

*Sagaritis oxylus* Cress.

At *Andropogon furcatus* consocias, 7.75 miles northwest of Thompson, June 30, 1928, one specimen. At *Spartina* consocias, 1 mile south of Amana, Aug. 13, 1927, two specimens. At *Carex socias*, 1 mile south of Amana, June 23, 1928, one specimen, and 7.75 miles northwest of Thompson, June 30, 1928, one specimen.

*Bathyplectes exigua* Cress.

At *Andropogon furcatus* consocias, 8 miles southeast of Britt, May 19, 1928, one specimen.

*Angitia obscura* Cress.

At all major communities except *Andropogon furcatus*—*Sorghastrum nutans* associates; common at *Andropogon furcatus*—*Spartina Michauxiana* associates, *Stipa spartea*—*Andropogon scoparius* association, *Andropogon scoparius*—*Bouteloua curtipendula* association, and *Carex* socies. Earliest adult, May 19, 1928; latest adult, Sept. 16, 1928.

*Pseuderipternoides porrectus* Cress.

At *Spartina* consocies, Lake Amana, Aug. 12, 1927, one specimen.

*Ceratogastra ornata* Say

Swept from flowers of *Solidago Riddellii*, 6 miles south of Washington, Aug. 24, 1928, three specimens.

*Cremastus graciliventris* Cush.

At *Spartina* consocies, 7.75 miles northwest of Thompson, Aug. 6, 1928, one specimen.

*Paniscus alaskensis* Ashm.

At *Andropogon furcatus*—*Sorghastrum nutans* associates, 1 mile south of Amana, Aug. 13, 1927, two specimens.

*Labrorychus prismaticus* Nort.

At *Carex* socies, 7.75 miles northwest of Thompson, June 30, 1928, one specimen.

*Dialges frontalis* var. *tricolor* Davis

At *Spartina* consocies, 8 miles southeast of Britt, May 19, 1928, one specimen.

*Tryphon communis* Cress.

At *Carex* socies, 1 mile south of Amana, June 23, 1928, one specimen.

*Polyblastus varitarsus* Grav.

At *Spartina* consocies, 2.5 miles south of Ames, May 20, 21, 1927, four specimens.

*Exyston variatus* Prov.

At *Spartina* consocies, 5 miles northwest of Buffalo Center, July 7, Aug. 6, 1928, two specimens.

*Syrphoctonus minimus* Cress.

At *Spartina* consocies, 8 miles southeast of Britt, July 6, 1928, one specimen.

*Zootrepes antennatus* Davis

At *Andropogon furcatus* consocies, 7.75 miles northwest of Thompson, Aug. 6, 1928, one specimen.

*Zootrepes compressiventris* Cress.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, common. Earliest adult, May 18, 1928; latest adult, Sept. 16, 1928.



*Phytodietus burgessi* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, Aug. 6, 1928, one specimen.

*Lissonota exilis* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 7.75 miles northwest of Thompson, June 30, 1928, four specimens; at *Carex* socies, same location and date, two specimens. Swept from flowers of *Cicuta maculata*, 5 miles northwest of Buffalo Center, July 7, 1928, one specimen.

*Lissonota frigida* Cress.

At *Stipa spartea*—*Andropogon scoparius* association, 5 miles northwest of Buffalo Center, July 7, 1928, one specimen.

*Epiurus pterophori* Ashm.

At *Polygonum amphibium* socies, 5 miles northwest of Buffalo Center, Sept. 16, 1928, one specimen.

*Tromatobia rufovariata* Cress.

At *Spartina* consocies, Lake Amana, Aug. 12, 1927, and June 23, 1928, two specimens, and 2.5 miles south of Ames, May 12, 20, 1927, two specimens.

*Gelis similis* Strickl.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Oak Grove State Park, July 25, 1926, two specimens.

*Gelis dimidiatus* Cress.

At *Andropogon furcatus* consocies, 8 miles southeast of Britt, July 6, 1928, one specimen, and at *Andropogon scoparius*—*Bouteloua curtipendula* association, Gitchie Manito State Park, July 24, 1928, one specimen.

*Cryptus persimilis* Cress.

At all major communities except *Andropogon furcatus*—*Sorghastrum nutans* associes; not numerous at any. Earliest adult, June 30, 1928; latest adult, Aug. 9, 1928. Swept from flowers of *Cicuta maculata*, 5 miles northwest of Buffalo Center, July 7, 1928, one specimen, and 5 miles east of Renwick, July 8, 1928, one specimen.

*Cryptus vinctus* Say

Swept from flowers of *Anemone canadensis* and *Erigeron ramosus*, June 23, 1928, three specimens.

*Glyphicnemis crassipes* Prov.

At *Carex* socies, 5 miles northwest of Buffalo Center, July 7, 1928, one specimen.

*Phygadeuon* sp. near *ovalis* Prov.

At *Andropogon furcatus* consocies, 8 miles southeast of Britt, July 6, 1928, one specimen, and at *Carex* socies, 5 miles northwest of Buffalo Center, July 7, 1928, one specimen.

*Centeterus tuberculifrons* Prov.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, Sergeant Bluff, July 26, 1928, one specimen.

*Phaeogenes fungor* Nort.

At *Andropogon scoparius*—*Bouteloua curtipendula* association, 4 miles northeast of Beloit, July 25, 1928, one specimen.

*Amblyteles jucundus* Brulle

Swept from flowers of *Cicuta maculata*, 5 miles northwest of Buffalo Center, July 7, 1928, two specimens.

*Amblyteles rubicundus* Cress.

At *Spartina consocias*, 2.5 miles north of Ames, May 2, 1928, one specimen.

*Leptopygus* sp. near *politus* Ashm.

At *Andropogon furcatus*—*Spartina Michauxiana* associates, common. Earliest adult, June 30, 1928; latest adult, July 7, 1928. Swept from flowers of *Cicuta maculata*, 5 miles northwest of Buffalo Center, June 30, July 7, 1928, four specimens.

## SUMMARY

The following list contains the more common and numerous species at each plant community.

*Andropogon scoparius*—*Bouteloua curtipendula* (beard grass—mesquite grass) association.

Order *Coleoptera*

*Disonychia latifrons*

Order *Hymenoptera*

*Angitia obscura*

*Stipa spartea*—*Andropogon scoparius* (porcupine grass—beard grass) association.

Order *Diptera*

*Tachydromia maculipennis*, *Philygria fuscicornis*

Order *Hymenoptera*

*Apanteles femur-nigrum*, *Angitia obscura*

*Andropogon furcatus*—*Sorghastrum nutans* (beard grass—wood grass) associates.

Order *Coleoptera*

*Chaetocnema confinis*

*Spartina* (slough grass) consocias.

Order *Coleoptera**Stenus punctatus*, *Longitarsus melanurus*.Order *Diptera**Aldrovandiella halterata*, *Dolichopus renidescens*, *Lonchoptera furcata*, *Scatella lugens*.Order *Hymenoptera**Rogas terminalis*, *Angitia obscura*, *Zootrephes compressiventris*.*Polygonum amphibium* (smartweed) socies.Order *Coleoptera**Disonycha pennsylvanica*.

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# CONSTANT TEMPERATURE AND HUMIDITY APPARATUS FOR USE IN THE EXPERIMENTAL STUDY OF INSECTS

T. A. BRINDLEY AND C. H. RICHARDSON

*From the Department of Zoology and Entomology, Iowa State College*

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Studies of insect ecology, physiology and toxicology have necessitated the development of apparatus in which the environment of an insect, especially the elements of temperature and relative humidity, can be closely controlled.

During the past two years considerable attention has been given by the Department of Zoology and Entomology at Iowa State College to the development of such apparatus for use in the study of insects. Success has been obtained in the use of a modified form of the constant temperature air bath designed by Clark<sup>1</sup> for studies on the hydrogen ion. The demand from research workers in entomology for detailed information regarding the construction of these boxes has urged us to place a description of them in the biological literature.

## CONSTRUCTION

Plate I shows the general view of the exterior of these boxes; Plates II and III illustrate the general internal structure.

The following dimensions are those of the box we have found to be of the most convenient size and shape: Outside construction: length 60 inches, width 30 inches, height 36 inches; doors to the main chamber; length 30 inches, width 19 inches and glass window 14 x 6 inches; door to the heating apparatus; length 30 inches, width 10 inches and glass window 14 x 3 inches. Inside construction: Dimensions of the main chamber; length to cone 36 inches, width 22 inches, height 24 inches; dimensions of heating chamber; length 7 inches, width 22 inches, and height 28 inches.

The walls of this cabinet are approximately 4 inches thick. Their construction is as follows: Next to the outside layer, which is panelled oak, there is a dead air space; after this a layer of any good fibrous insulating board such as Maizewood or Cellotex, followed again by a three-fourths inch dead air space, a layer of insulating board, another three-fourths inch dead air space, and a final layer of insulating board. This type of insulation has proved effective, often preventing the temperature of the box from reaching room temperature after the heating units have been shut off for several hours. Cases have been observed where the loss of heat from the box, when the heating units were turned off, was less than one degree C. in three to five hours.

The United States Bureau of Standards<sup>2</sup> shows that the heat loss per square foot per hour under a temperature gradient of 1° F. per inch of

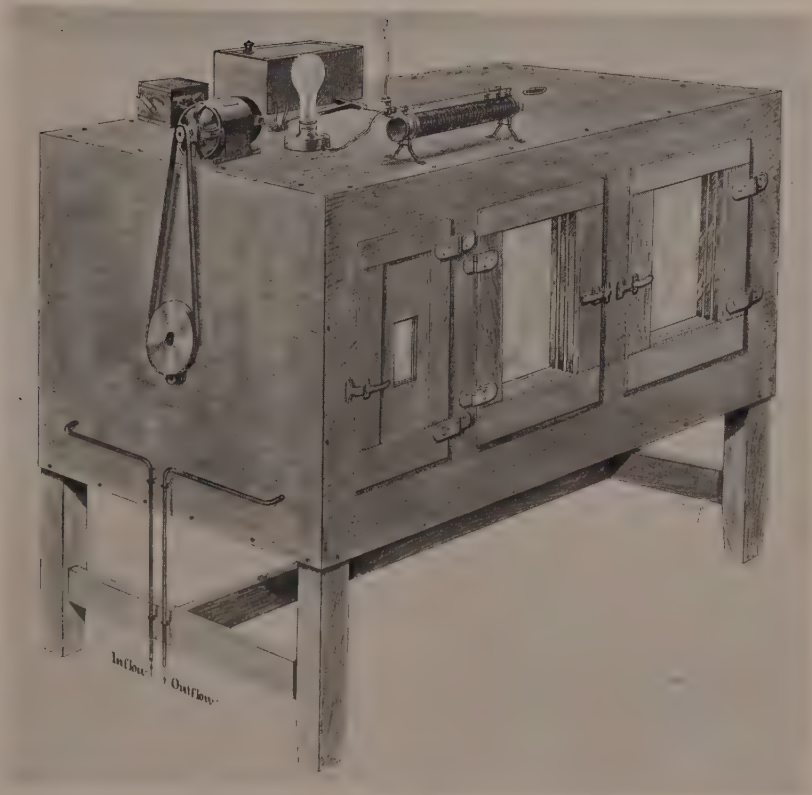
<sup>1</sup>Clark, W. Mansfield. The determination of hydrogen ions. Williams and Wilkins Co., Baltimore, pp. 166-174. 1920.

<sup>2</sup>Burgess, George K. The thermal insulation of buildings. U. S. Dept. of Commerce. Circ. Bur. of Standards, 376. 1929.

## PLATE I

View of the exterior of constant temperature control cabinet

PLATE I

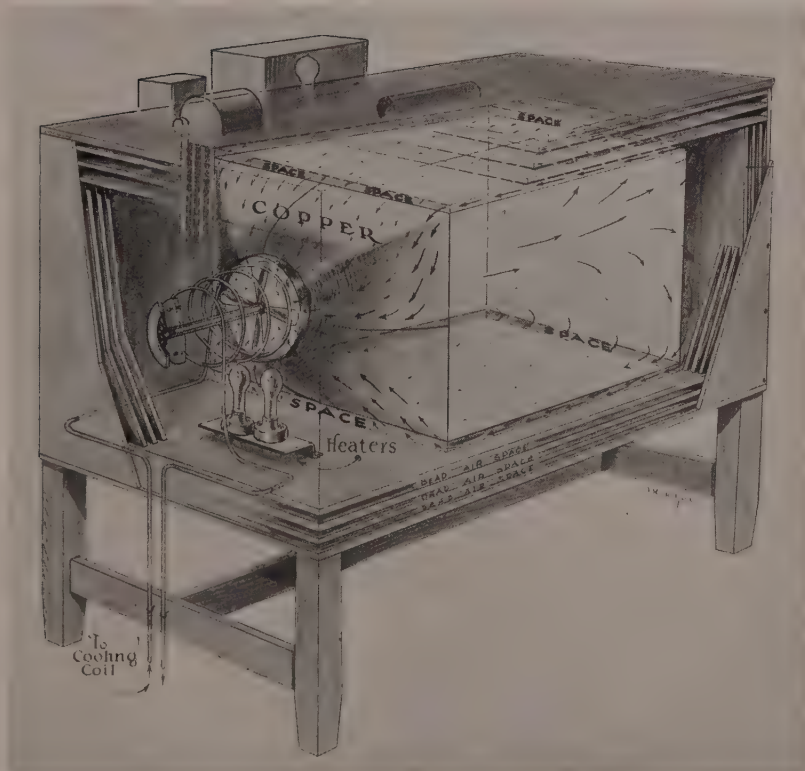


## PLATE II

View of the internal structure of constant temperature control cabinet--false top and  
bottom construction



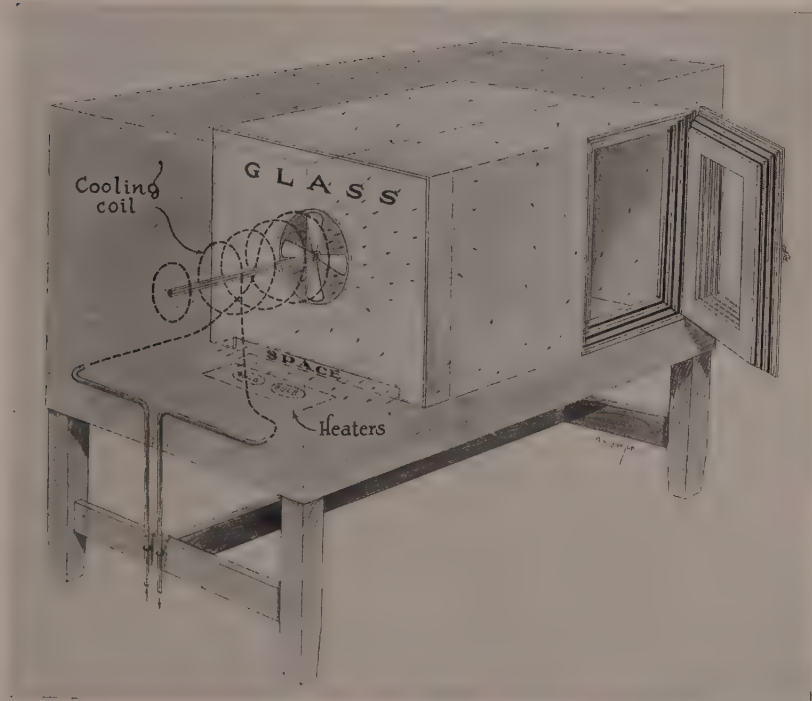
PLATE II



## PLATE III

View of the internal structure of constant temperature control cabinet—diaphragm type  
of construction

PLATE III







thickness of fiber board lies between 0.25 and 0.35 B. t. u., whereas the ideal insulator, still air, permits a loss of 0.2 B. t. u.

As shown in Plate II, the interior of the box is provided with a false top and bottom and a cone-shaped diaphragm of copper which separates the main chamber from the heating units. The false top and bottom are placed two inches from the top and bottom of the box, respectively, and two inches from the wall at the end opposite the diaphragm. This type of construction permits an efficient circulation of air resulting in a uniform temperature throughout the main chamber.

A box of the plane diaphragm type of construction is illustrated in Plate III. In this cabinet the diaphragm consists of any suitable material (glass, metal or insulating board) with a round hole for the fan and a space of three inches at the bottom to allow a return circulation of air over the heating units. This type of construction gives more working space than in the cabinet described above, but temperature control is not quite so uniform in all parts of the chamber.

Three types of lining were tried in the boxes, namely, glass, copper, and insulating board coated with lead paint and lacquer. The glass and copper lined boxes were designed for close humidity control or the use of toxic gases, but showed no better temperature control than the one lined with insulating board. The relative merits for humidity control of the various lining materials is discussed below.

#### TEMPERATURE CONTROL APPARATUS

The temperature in the boxes is controlled by a metastatic thermoregulator. This regulator consists of a thermometer-like tube with two platinum contacts sealed into the capillary near the top. The top of the tube is enlarged to form a bulb into which the capillary, bent in the form of a goose-neck, extends. The entire capillary is filled and the bulb partially filled with mercury. Before sealing, the latter is filled with dry inert gas. This instrument may be purchased in various sizes from several companies dealing in temperature control apparatus.

For close regulation of temperature, a circuit in which a small direct current operates a sensitive relay through the thermoregulator, is generally most effective. The relay controls the heating units. Two kinds of relays have been used successfully in these circuits, for example, a sensitive relay with large metallic contacts and a sensitive mercury relay. Both operate on as little as four milliamperes of direct current at about three volts. The former requires some attention to the contacts and after long service the points which carry the current to the heating units must be replaced. The contacts will in time become foul and must be cleaned with fine sandpaper (00 grade) every six to eight weeks when the cabinet is in continuous operation. With this attention the relay will give excellent service. The mercury type makes and breaks the heating circuit through mercury enclosed in a glass tube and therefore is free from trouble caused by oxidation at the contacts. It is somewhat less expensive than the relay first mentioned, but our experience with it has been of shorter duration.

The direct current for the thermoregulator circuit may be supplied by a 6 volt storage battery or small rectifying transformer which reduces the 110 or 220 volt alternating current to 8 volts direct current. The trans-

former furnishes sufficient current and voltage to operate two constant temperature cabinets of the size described above. It is lower in price and much more dependable than a storage battery. We have hesitated to employ vacuum tube rectification because our requirements call for continuous operation and the life of most vacuum tubes is less than ten thousand hours.

The heating elements used are Mazda electric light bulbs which are painted black, if light within the cabinet is not desired. The number and wattage of the bulbs depends upon the temperature required and the gradient between outside and inside temperature. We have obtained best results by using bulbs of such wattage that the heaters are switched on and off at short intervals of approximately equal length. It is safer to use several bulbs instead of one, in order to guard against the burning out of a single heating unit. Bulbs vary considerably in heat lag and only those should be selected which show a negligible lag under operating conditions.

As efficient temperature control is dependent upon good air circulation within the cabinets, considerable attention has been given to the selection of motors and the construction of fan shafts. It is advisable to choose a motor of rugged construction and long life, because the failure of air circulation during an experiment may prove disastrous. We have used three types: A small fan motor (110 volts, A. C. 60 cycles), a small high-speed motor (H. P.  $1/15$ ,  $50^{\circ}$  C., 110 volts A. C., 60 cycles, 1.27 amperes, 3,000 r. p. m.), and a large slow-speed motor (H. P.  $1/4$ ,  $55^{\circ}$  C., 110 volts A. C., 60 cycles, 1,080 r. p. m.). All have given good service. Under ordinary conditions, the motor is run at a speed sufficient to give a perceptible movement of air, but not an air blast. When a wet and dry bulb humidity reading is desired, however, it is necessary that the air move at a higher rate and for this purpose a motor of moderately high speed is preferable. A motor rating 2,000 to 3,000 r. p. m. in circuit with a permanent resistance (lamp) and a sliding rheostat will give almost any desired rate of air movement.

The shaft which carries the fan should be equipped with Babbitt metal or other suitable bearings, and enclosed in a metal housing which is securely bolted to the side of the cabinet (see Plate II). An eight inch fan with propellor-shaped blades is placed at the end of this shaft. Ordinary house fans with nearly flat blades are not suitable because the air is driven laterally instead of directly forward across the cabinet. However, if the blades are metal they may be bent to a concave shape and then will function very well.

For the maintenance of temperatures between  $20^{\circ}$  C. and  $25^{\circ}$  C. in most parts of the United States in the summer time some form of artificial cooling is necessary. At Ames, Iowa, tap water at  $15^{\circ}$  C., circulating through a coil of copper tubing 15 feet in length, has been sufficient to maintain  $25^{\circ}$  C. in the cabinet against outside room temperatures as high as  $35^{\circ}$  to  $40^{\circ}$  C.

Actual operating temperatures taken in different parts of cabinets of various construction are shown in table 1. They were made when the cabinets had one or two ports open in the top. Constant renewal of outside air through openings to the exterior may be an important consideration when the cabinet is filled with a large quantity of living material.

TABLE 1. *Temperature control in constant temperature air baths of different construction\**

Type of construction	Diaphragm		False top and bottom			
Lining material	Glass		Copper**		Cellotex	
Location of thermometers	Box temp.	Room temp.	Box temp.	Room temp.	Box temp.	Room temp.
Instrument end						
Upper right hand corner	25.1	22.0°C.	25.3	31.0°C.	30.0	21.5°C.
Upper left hand corner	25.1	"	25.2	"	30.0	"
Lower right hand corner	25.1	"	25.3	"	30.0	"
Lower left hand corner	25.1	"	25.2	"	30.1	"
Middle, near thermoregulator	25.1	"	25.3	"	30.0	"
Opposite end						
Upper right hand corner	25.2	"	25.3	"	30.0	"
Upper left hand corner	25.2	"	25.3	"	30.0	"
Lower right hand corner	25.1	"	25.4	"	30.0	"
Lower left hand corner	25.0	"	25.3	"	30.0	"
Middle, near thermoregulator	25.1	"	25.3	"	30.0	"
Center of box	25.1	"	25.3	"	30.1	"

\*Observations made with a standard thermometer to the nearest 0.1 of a degree. Two or more ports open.

\*\*Cooling system in operation.

#### HUMIDITY CONTROL

Humidity can be controlled with fair accuracy in these cabinets by means of saturated solutions of certain salts containing an excess of the salt.

TABLE 2. *Humidity control in constant temperature air baths of different construction*

Type of construction		Diaphragm		False top and bottom			
Lining material		Glass (25°C.)		Copper (25°C.)		Fiber board (28.3°C.)	
Time		T <sup>1</sup> —T <sup>2</sup>	R.H.	T <sup>1</sup> —T <sup>2</sup>	R.H.	T <sup>1</sup> —T <sup>2</sup>	R.H.
1st day	A. M.	7.2	70	7.3	70	7.7	71
	P. M.	7.2	70	7.3	70	7.7	71
2nd day	A. M.	5.9	75	7.3	70	8.1	69
	P. M.	5.9	75	7.3	70	9.0	66
3rd day	A. M.	6.3	74	7.6	69	7.7	71
	P. M.	6.3	74	7.6	69	8.1	69
4th day	A. M.	6.8	72	7.2	70	8.1	69
	P. M.	6.3	74	7.2	70	7.7	71
Average Relative Humidity		73		70		70	

T<sup>1</sup> = Temperature of dry bulb; T<sup>2</sup> = Temperature of wet bulb.

Between 20° and 40° C. a saturated solution of sodium chloride will fill a space with sufficient water vapor that at equilibrium the relative humidity is very close to 75 per cent. Tests in copper lined, glass lined and fiber-board lined cabinets with a saturated solution of sodium chloride gave average relative humidities of 70 to 73 per cent. The exposed area of the salt solution was 50 square inches. The tests are detailed in table 2.

It should be pointed out that our requirements call for continuous operation. One cabinet is in its third year of operation, during which time it has not been shut down for repairs for more than ten days. Such performance implies good construction and reliable electrical equipment. Although these requirements have been met in a fair degree by the apparatus described above, improvements are desirable, particularly in the thermoregulator, which after long usage may need replacement.



# A PRELIMINARY REPORT ON INTESTINAL PROTOZOA OF WHITE GRUBS (PHYLLOPHAGA SPP.—COLEOPTERA)

BERNARD V. TRAVIS AND E. R. BECKER

*From the Entomology Section, Iowa Agricultural Experiment Station*

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Protozoan parasites of the white grubs have been mentioned many times in entomological literature. Some authors (e.g., Forbes, 1916, and Davis, 1919) name the protozoa as the causative agents in certain white grub (*Phyllophaga* spp.) diseases. No author gives specific information, however, concerning the class, order, genus or species of the protozoa which are supposed to be incriminated.

The protozoa treated in this paper were obtained from the rectal contents of hibernating grubs (Brood A) of the genus *Phyllophaga* Harris taken near Leon, Iowa, and were examined in both the living state and stained preparations. Smears of the infected rectal contents were fixed in warm Schaudinn's fluid and stained with Heidenhain's iron-hematoxylin. Measurements, as stated for length, were from the posterior to the anterior tips of the body, not including the flagella. *Amoebae* and *Monocercomonas* grew profusely in Ringer's egg-albumen medium at room temperature with a pH of 7.4. The formula employed is as follows:

Sodium chloride .....	0.80 part
Calcium chloride (anhydrous) .....	0.02 part
Potassium chloride .....	0.02 part
Sodium bicarbonate .....	0.02 part
Distilled water .....	100 parts
Fresh egg albumen .....	1 part

Only members of the classes *Mastigophora* and *Rhizopoda* were represented in the fauna of the white grub. The genera *Polymastix*, *Monocercomonas*, *Eutrichomastix*, and *Embadomonas* constituted the *Mastigophora*. Two amoeba belonging to the genus *Vahlkampfia* were found. All species were found, at times, to be infected with coccid parasites (figs. 5, 17, 21).

None of the forms noted appeared to be pathogenic to the grubs, for all of them seemed to be harmless commensals feeding upon bacteria only. Two free living protozoa were encountered inside the grubs, but only one specimen of each was observed. One of these was a *Rhizopoda* of the genus *Arcella* (fig. 36) and the other a flagellate belonging to the genus *Allan-  
tion* (fig. 37).

## POLYMASTIX Bütschli

Grassi (1881) described a flagellate from the rectum of *Melolontha* which he called *Trichomonas melolonthae*. Bütschli (1884) later created the genus *Polymastix* for this organism. Other workers have recorded this species from other hosts: Künstler (1882, 1883), from *Rhizotrogus* and

Oryctes; Hamburger, 1891), from Cetonina; Mackinnon (1912, 1914), from Tipula; Franca (1913), from Oryctes; Grassé (1926), from Hanne-ton; and Zelif (1930), from Anomala. Grassé (1926) described *P. legeri* from Glomeris, and Hasselmann (1927) described *P. nitidus* from Rhinocricus.

#### DIAGNOSIS OF THE GENUS

The body is pyriform with four anterior flagella of unequal length and originating in pairs from two large blepharoplasts situated at the anterior tip of the body. A slender axostyle and a parabasal body have their origin in one of the blepharoplasts. The nucleus is anterior, with chromatin material in the form of a large karyosome. The body is covered with long rod-shaped bacterial ectoparasites. Members of this genus are parasites of insects and myriapods.

#### *Polymastix melolonthae* (Grassi) Bütschli

Synonymy: *Trichomonas melolonthae* Grassi (1881); *Costifera* Grassi (1888)

The body is somewhat spindle-shaped, but with a rounded anterior end and a pointed posterior end. This species is slender and wrinkled (fig. 1). Grassi (1882) figured specimens with forked tails, and a few such forms are present on our stained slides (fig. 2). Mackinnon (1914) and Zelif (1930) figure individuals with the posterior end rounded. Similar specimens are found on stained slides, but these are only at the edges of the smears and seem to have been distorted in the smearing process.

The periplast is thick and ridged. Living specimens present an evenly striated appearance, but after staining the striations are seen to be composed of deeply-staining rods which are discontinuous and fall into groups inclined at various angles (fig. 6). These rods fray off when the animal dies, and often in the staining process (fig. 7). Grassi (1882) thought they might be trichocysts. Franca (1882, 1883) called them bacteria, but said they were accidental. Hamburger (1911) described both rods and striations and compared the striations to those on *Euglena*. Mackinnon (1914) called them thickened ribs. Grassé (1926) described them as bacteria of the genus *Fusiformis*.

The four flagella and the slender axostyle have their origin in the two large blepharoplasts situated at the anterior tip of the body. The two flagella originating in the blepharoplast which gives rise to the axostyle are short; the other blepharoplast gives rise to one long flagella and one of medium length. The short flagella are slightly longer than the body; the long one is noticeably longer. The axostyle arises thread-like from a blepharoplast, thickens as it curves around the nucleus, and then tapers gradually until it disappears from view near the middle of the body (figs. 2, 3, 4); but in some individuals it is visible throughout the entire length of the body (fig. 1).

The nucleus is round with a thin, indistinct membrane. The chromatin material is in the form of a large centrally or eccentrically placed karyosome.

A parabasal body is often present, and its connection with a blepharoplast, as noted by Grassé (1926), can be demonstrated (fig. 3).

The cytoplasm is filled with large vacuoles, but no food material is observable.

The size varies from  $14.3\mu \times 2.3\mu$  to  $22.8\mu \times 5.7\mu$ .

### *Polymastix phyllophagae* n. sp.

The spindle-shaped body is not wrinkled or forked, and it is wider in proportion to its length than *P. melolonthae*. The periplast is rigid and is covered with ectoparasitic bacteria.

The blepharoplasts give rise to flagella of the same relative lengths as in *P. melolonthae*, and to a long thread-like axostyle which differs from that of *P. melolonthae* in that it typically extends the full length of the body, following a wavy course. The nucleus usually does not have a distinct membrane (fig. 7). A few specimens have a pear-shaped nucleus (figs. 5, 6). K nstler (1882) and Mackinnon (1914) described a cytostome located between the basal granules. Grass  (1926) thinks this structure is an optical illusion caused by the refraction of light by the thicker protoplasm at the anterior end. Hasselmann (1927) finds a prominent cytostome in *P. nitidus*.

The parabasal apparatus is like that in *P. melolonthae* (fig. 9). The cytoplasm is filled with vacuoles, but no food material is observable.

The size varies from  $4.75\mu \times 3.8\mu$  to  $12.4\mu \times 5.4\mu$ .

### MONOCERCOMONAS Grassi

Grassi (1879a) divided the genus *Monocercomonas* into four sub-genera: (1) *Monocercomonas*, (2) *Trichomonas*, (3) *Retortamonas*, and (4) *Schedoacercomonas*. In the genus *Monocercomonas* he included flagellates from man, guinea-pig, snake, frog, mouse, and lizard. Grassi (1881) later removed all of these to the genus *Trichomonas* except *M. coronellae*, which is, then, the type species of the genus. This species is synonymous with *Bodo colubrorum* Hammerschmidt (1884), which, however, Grass  (1926) says is *Eutrichomastix*.

There now remains the *Monocercomonas* of insects. Grassi (1881) applied the name *Monocercomonas insectorum* to two flagellates, *Schedoacercomonas gryllotalpae* of the mole cricket and *S. melolonthae* of the cockchafer. Grass  (1926), however, finds no forms with four flagella in the mole cricket, but he finds a form with six flagella which he places in the genus *Hexamastix* Alexeieff (1912). *S. melolonthae* has four flagella; so if "*S. gryllotalpae*" has six flagella, both can not belong to the same genus. This bewildering confusion has caused Grass  (1926) in desperation to re-tain the genus *Monocercomonas*; for, he says, "j'estime que le rem de savait pire que le mal."

### DIAGNOSIS OF THE GENUS

These are small flagellates with four sub-equal flagella arising in pairs from two blepharoplasts that are connected with a short rhizoplast. From one of the blepharoplasts there originates a filamentous axostyle and a parabasal apparatus. The nucleus is large with the chromatin material in the form of a large karyosome. The members of this genus are parasites of insects and myriapods.



**Monocercomonas melolonthae** Grassi

Synonymy: *Schedoaceromonas melolonthae* Grassi 1879; *Monocercomonas insectorum* Grassi 1881.

This species is easily recognized by the enormous nucleus with a large karyosome and chromatin incrustation on the nuclear membrane (fig. 32). The blepharoplasts and their connecting rhizoplast are often pressed closely against the nuclear wall (fig. 32), in which case the nucleus is likely to be pear-shaped. The axostyle may be absent, but when present it appears as in fig. 35. A few specimens have the "siderophilous granules" (fig. 33) that Mackinnon (1912) described, but no parabasal apparatus like that described by Grassé (1926) in which a large deeply-staining body originates in the blepharoplast and curves around the nucleus. The stained cytoplasm is finely vacuolated, but no vacuoles are observed in living specimens unless they are degenerating.

The sizes varies from  $5.7\mu \times 4.8\mu$  to  $9.5\mu \times 5.7\mu$ .

**EUTRICHOMASTIX** Kofoid and Swezy

Hammerschmidt (1884) described from a snake a flagellate, *Bodo colubrorum*, which is synonymous with *Monocercomonas coronellae* Grassi (1879). Blochmann (1884) described *Trichomastix lacertae*, which is synonymous with *M. coronellae*, but Vollenhoven (1878) had already given this generic name to an insect. Kofoid and Swezy (1915) consequently created the genus *Eutrichomastix* as a substitute. Dobell (1907) described *E. serpentis* from the rectum of a boa and found that in culture media this species developed into forms indistinguishable from *Trichomonas*. Chatton (1920) demonstrated that *T. caviae* could be made to assume the *Eutrichomastix* form by controlling the condition of culture media. (This behavior was reversible.) Grassé (1926) concludes from these and similar works that *Eutrichomastix* should be united with *Trichomonas*, but this procedure is doubtful since *Eutrichomastix* and *Trichomonas* are not known to commonly change form in their hosts. Mackinnon (1910) described *Trichomastix trichopterae* from tipulid larvae. Tanabe (1926) described *E. passali* from *Passalus*. Cunha and Muniz (1927) described *E. aguti* from *Dasyprocta*. Bernstein (1928) described *E. termitis* from the termite.

## DIAGNOSIS OF THE GENUS

The body is pyriform. There is an anterior blepharoplast from which originate four flagella—three anterior and one posterior—and a well-developed axostyle. The nucleus is anterior and spherical. The members of this genus are principally parasites of insects and snakes.

**Eutrichomastix passali** Tanabe

This species (figs. 10, 11, 12) was not observed in the living state, but was found to be present in prepared slides from one grub.

The body is pyriform and provided with four unequal flagella—three anterior and one trailing—all arising from a large blepharoplast at the anterior tip of the body. The anterior flagella are a little longer than the body, the trailing flagellum much longer. The lightly staining axostyle is



straight or slightly curved. It projects slightly and ends in a point. The nucleus is vesicular with diffuse chromatin. The nuclear wall is thin, but rarely indistinguishable. The body is finely vacuolated. Small siderophilous granules are distributed about the nucleus.

The size varies from  $6\mu \times 2.9\mu$  to  $9.5\mu \times 5.7\mu$ .

#### *Eutrichomastix phyllophagae*, n. sp.

This flagellate is abundant in grub worms. The organism is pyriform and shows marked variations in both size and shape. The protoplasm stains heavily with hematoxylin and is filled with bacteria in various stages of digestion, although no cytostome was observed. It is frequently so infected with coccus parasites that the internal structures are obscured (figs. 17, 21). The protoplasm often passes to one end of the axostyle and gives the organism a distorted appearance (figs. 20, 21, 22). The axostyle is long and well developed. There are two types of axostyles—one thick and light-staining (fig. 20), and the other deeply staining and thread-like (fig. 22). The axostyle may be twice the length of the body in some forms (fig. 23). The anterior flagella are slightly longer than the body, while the posterior flagellum is often three times the length of the body. Living specimens have their axostyle attached to masses of bacteria, fat cells, fat globules, and debris. The trailing flagellum entangles itself in debris and, like the cytoplasm around the tip of the axostyle, seems viscous.

The nucleus is round and is located in any part of the anterior half of the body. The nuclear wall is thin and often indistinguishable. The chromatin is contained in a large spherical karyosome.

The size varies from  $5.4\mu \times 2.8\mu$  to  $9.5\mu \times 5.3\mu$ .

#### *EMBADOMONAS* Mackinnon

Mackinnon (1911) created this genus for a protozoon, *E. agilis*, which she found in trichopterid larvae. Later, in 1912, she described *E. alexeieffi* and *E. agilis* from tipulid larvae. Wenyon and O'Connor (1917) described *E. intestinalis* from man; Fonseca (1917), *E. wenyoni* from Cebus; Faust and Wassel (1921), *E. sinensis* from man; and Brug (1922), *E. belostomae* from *Belostoma*.

#### DIAGNOSIS OF THE GENUS

The members of this genus are slipper-shaped, and have a large cytostome with a chromophilic border. Two flagella, one anterior and one in the cytostome, arise from separate blepharoplasts. The nucleus is spherical and anterior with the chromatin arranged in a large karyosome, several granules, or in a crescent-shaped mass against the periphery of the nucleus. The genus includes parasites of insects and a number of mammals.

#### *Embadomonas phyllophagae*, n. sp.

This organism was very abundant in one white grub. The adult forms (fig. 15) are typically slipper-shaped, but there is much variation in size and the young forms are typically pear-shaped (figs. 13, 16). The body is slightly robust. The anterior end is slightly curved dorsally (fig. 15) and

to one side, so that the cytostome is directed antero-ventrically. The bending and twisting of the body, with the flagellar action, causes the animal to have a spiral movement in a liquid medium. The flagella are of the same diameter. The anterior flagellum is slightly shorter than the body. The cytostomal flagellum is seldom longer than the cytostome, and stains lighter than the anterior one.

The size varies from  $4.75\mu \times 4.17\mu$  to  $9.5\mu \times 4.9\mu$ .

#### *VAHLKAMPFIA* Chatton and Lalung-Bonnaire

In live material taken directly from the grub, two types of amoeboid movement were observed. Some amoebae showed the monopodal form with the typical limax movement. Others were of the type with many pseudopods.

A large and a small form were observed on stained slides. The smaller form (fig. 27, 28), is characterized by deeply-stained pseudopods. The endoplasm contains vacuoles with bacteria in various stages of disintegration through digestion. The nucleus is vesicular with a large central karyosome. In the optical plane about ten linin fibers running from the karyosome to the nuclear wall can be observed. The cyst has the same type of nucleus, and is filled with chromophilic granules.

The trophozoites vary in size from  $6.8\mu \times 5.7\mu$  to  $9.6\mu \times 7.6\mu$  and the cysts measure from  $6.3\mu$  to  $7.4\mu$ .

The larger form (figs. 25, 26) is much like an amoebae which Mackinnon (1912) figured and referred to as *Entamoeba* with the *Amoeba limax* type of nucleus. In 1914a she further described it, but gave it no name except *Vahlkampfia* sp. No living individuals were noted, but two were found on stained slides. The endoplasm is filled with large vacuoles and bacteria. The nucleus is large and vesicular, with a central karyosome filling about half the nucleus. Some dark staining granules are distributed between the karyosome and the nuclear wall.

The sizes of the two observed specimens were  $13.6\mu \times 9.5\mu$  and  $13.8\mu \times 8.9\mu$ .

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#### DESCRIPTION OF FIGURES

All figures, unless otherwise indicated, are drawn to scale x 3,000 from specimens fixed in Schaudinn's fluid and stained by the Heidenhain iron-hematoxylin method.

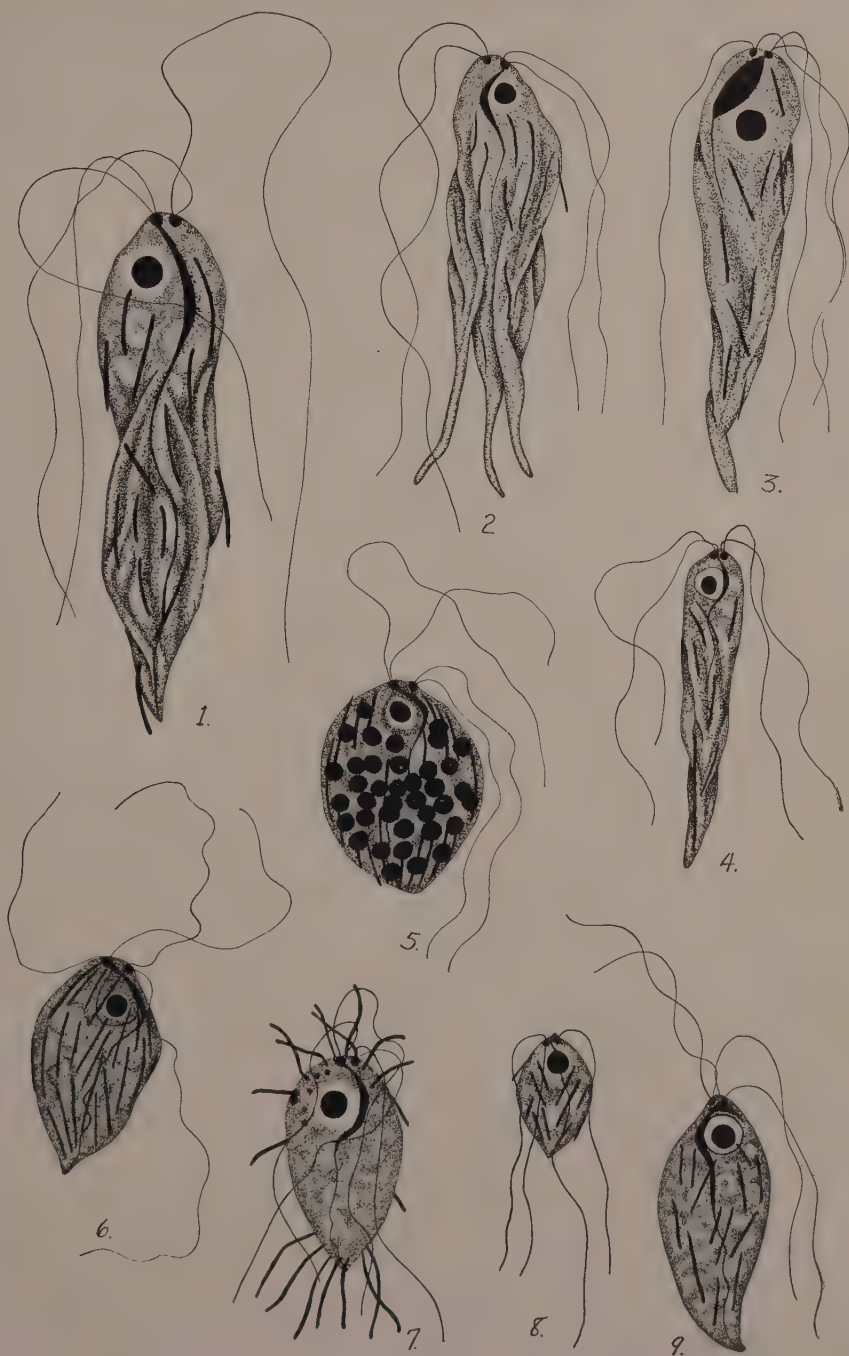
##### *Polymastix melolonthae* (Grassi) Bütschli

- Fig. 1. Trophozoite with long axostyle.  
Fig. 2. Trophozoite with split posterior end.  
Fig. 3. Trophozoite with parabasal apparatus.  
Fig. 4. Young form.

##### *Polymastix phyllophagae*, n. sp.

- Fig. 5. Parasitized trophozoite.  
Fig. 6. Trophozoite with the typical long axostyle.  
Fig. 7. Trophozoite with bacteria fraying off.  
Fig. 8. Young form.  
Fig. 9. Trophozoite with parabasal apparatus.





*Eutrichomastix passali* Tanabe

Fig. 10-12. Trophozoites.

*Embadomonas phyllophagae*, n. sp.

- Fig. 13. Young form.
- Fig. 14. Trophozoite.
- Fig. 15. Trophozoite with typically pointed end.
- Fig. 16. Young form.

*Eutrichomastix phyllophagae*, n. sp.

- Fig. 17. Trophozoite filled with *Cocceus* parasites.
- Fig. 18. Dividing form.
- Fig. 19. Young form.
- Fig. 20. Distorted form with large axostyle.
- Fig. 21. Trophozoite with small axostyle.
- Fig. 22. Young form (degenerating).
- Fig. 23. Young form with long curved axostyle.
- Fig. 24. Trophozoite.



Travis del.

*Vahlkampfia* Chatton and Lalung-Bonnaire

Fig. 25-26. Large form.

Fig. 27-28. Small form.

Fig. 29. Cyst.

*Monocercomonas melolonthae* Grassi

Fig. 30. Trophozoite with blepharoplasts removed from the nucleus.

Fig. 31. Trophozoite with abnormal axostyle.

Fig. 32. Trophozoite with eccentric karyosome.

Fig. 33. Trophozoite with siderophilous granules.

Fig. 34. Dividing form.

Fig. 35. Adult form with typical axostyle.

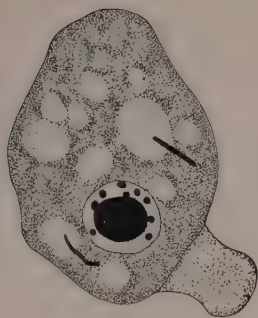
*Arcella* sp.

Fig. 36. Arcella shell (side view) x 1000.

*Allantion* sp.

Fig. 37. Trophozoite (soil flagellate).





25.



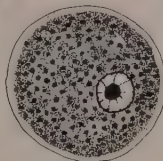
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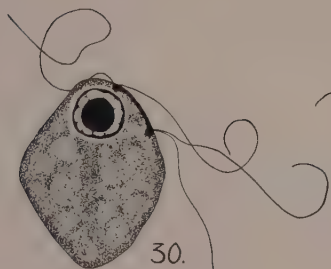
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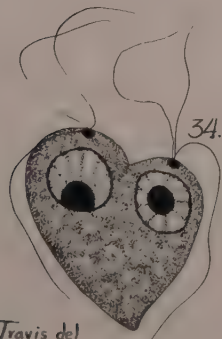
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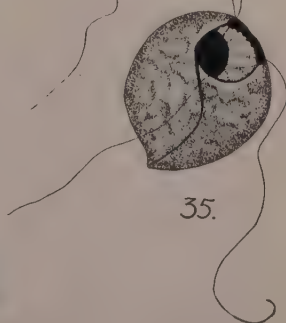
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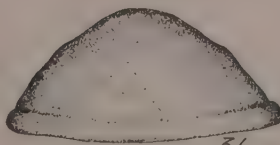
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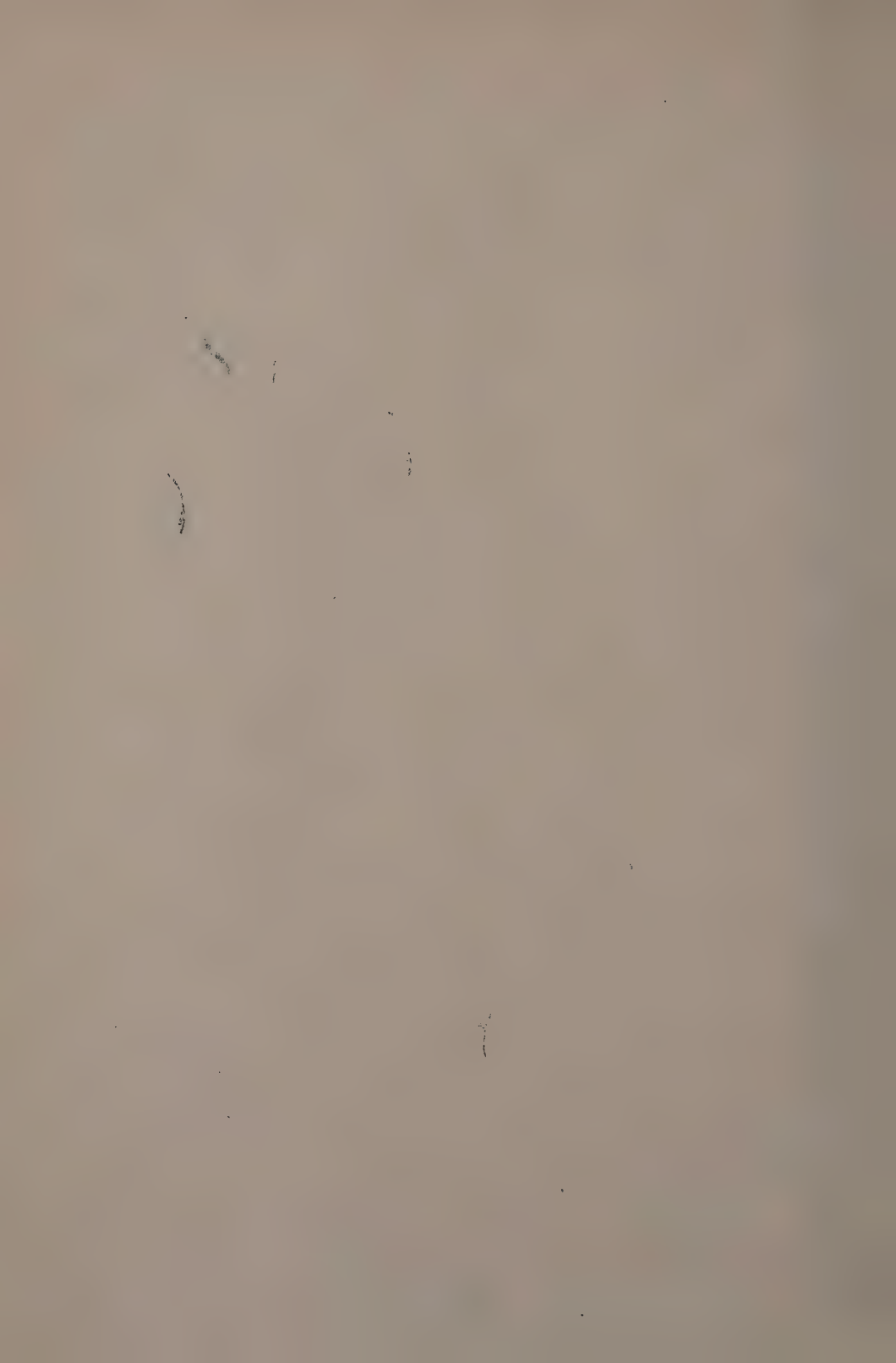
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35.



36.



# A MICROSCOPIC STUDY OF CELLULOSE PULPS PREPARED FROM PARENCHYMATOUS AND VASCULAR TISSUES OF THE CORNSTALK

FLORENCE E. HOOPER

*From the Chemistry Department of Iowa State College*

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The properties of paper are in part determined by the properties of the individual cells in the pulp from which it is prepared. For this reason, a microscopic study of cellulose pulps prepared from the tissues of the cornstalk is of practical interest. For the sake of comparison, the present study was confined to properties which had been previously studied in wood pulps.

## METHOD AND RESULTS

### MEASUREMENTS OF THE CELLS OF THE PULP

Measurements were made, by means of an eyepiece micrometer, on cells of pulps prepared by a modification of the de Vain's process. The results are presented in table 1. The corresponding data for a number of broad-leaved woods are shown in table 2. It may be seen from these tables, that although the dimensions of a typical broad-leaved wood fiber are less than those of a typical cornstalk fiber, the ratio of length to width is of the same order in the two cases.

TABLE 1. *Measurement of cells of cornstalk pulp*

Type cell	Length in —				Width in —				Average ratio: length/width
	No. of cells measured*	Mm. Average	Mm. Maximum	Mm. Minimum	No. of cells measured*	Mm. Average	Mm. Maximum	Mm. Minimum	
Fiber	202	55.52	23.15	1.01	177	0.167	0.03	0.07	33
Pith	199*	1.43	2.66	0.40	+				1 <sup>+</sup>

\*Three or four typical cells were measured on each of a large number of mounts.

\*Since it is impossible to differentiate between length and width in pith cells, all measurements were used in the construction of one average value.

The formation of a mat is caused by the interlacing of fibers, and to the cohesion of the surfaces of the cellulose cells. The extent to which interlacing will occur will largely depend upon the length to width ratio of the fibers. In view of this fact, it is not surprising that mats of similar properties may be prepared from cornstalk and wood fibers. Obviously, in the case of cells with a length-to-width ratio equal to one, mat formation will

TABLE 2. *Dimensions of fibers of some broad-leaved woods\**

	Average length in —	Average width in —	Average ratio length/ width
Beech ( <i>Fagus grandifolia</i> )	1.13	0.022	51
Large-toothed aspen ( <i>Populus grandidentata</i> )	1.08	0.028	39
Aspen ( <i>Populus tremuloides</i> )	1.15	0.032	36
Tupelo gum ( <i>Nyssa aquatica</i> )	1.85	0.066	28
Red alder ( <i>Alnus rubra</i> )	1.23	0.027	45
Sycamore ( <i>Platanus occidentalis</i> )	1.57	0.024	65
Red maple ( <i>Acer rubrum</i> )	0.93	0.020	46
Buckeye ( <i>Aesulus flava</i> )	0.62	0.020	31
Cucumber magnolia ( <i>Magnolia acuminata</i> )	0.86	0.029	29
Umbrella ( <i>Magnolia fraseri</i> )	1.08	0.027	40
Yellow poplar ( <i>Liriodendron tulipifera</i> )	1.14	0.029	39
Red gum ( <i>Liquidamber styraciflora</i> )	1.55	0.031	50
Black gum ( <i>Nyssa sylvatica</i> )	1.68	0.026	65
American elm ( <i>Ulmus americana</i> )	1.35	0.019	71
Paper birch ( <i>Betula papyrifera</i> )	1.17	0.025	47

\*Constructed from experimental data reported by Sutermeister (2).

These measurements are for the fibers rather than for pulp cells. It seems unlikely that this would make any great difference in either the individual measurements or in the ratio.

not be caused by interlacing of fibers. Such a mat would be expected to differ in properties from one of the interlaced type. A comparison of the papers prepared from pulps prepared from parenchymatous and vascular tissues of the cornstalk show that the facts are in agreement with this prediction.

#### DISINTEGRATION OF THE CELLS OF THE PULPS BY PHOSPHORIC ACID-ABSOLUTE ALCOHOL TREATMENT

Delignified vascular bundles of the cornstalk were dehydrated in alcohol, subjected to swelling and shrinking by treating alternately with phosphoric acid and absolute alcohol, and finally mounted in acid. Sixty-eight, seventy-six and eighty-five per cent acids were used. The time allowed for the individual treatments varied from three to fifteen minutes. The swollen cells were subjected to slight pressure by pressing on the cover glass, and were examined under the microscope.

Typical fiber or tracheid cells were first examined. Under the described treatment the cellulose wall separated into several concentric layers (Pl. I, fig. 1.) Solution of the various layers was preceded by striation and separation into spirally wound fibrils. The fibrils of the outer layer form an angle of approximately 90 degrees to the long axis of the cell (Pl. I, fig. 2), while the fibrils of the inner layers are wound at an angle of 30 to 45 degrees. (No satisfactory photomicrographs were obtained.)

These observations agree with those of Ritter (1) on delignified elm fibers. He used this difference in orientation of the fibrils of the inner and outer layers to explain the difference in swelling exhibited by them. Similar behavior was observed in the cornstalk. Thus, as the outer wall dissolves away the inner layers rapidly swell outward with constriction where



the outer layer is still intact (Pl. I, fig. 3). This apparent structural similarity of wood and cornstalk fibers suggests that the ability of the two fibers to stand stress and strain will be of the same order of magnitude.

An attempt to study the structure of the walls of parenchymatous pulp cells by the same method was unsuccessful. Crumpling and complete solution occurred so rapidly that no observations could be made. It seems possible that this difference in behavior may be because of the relative thinness of these walls rather than to any structural difference. Regardless of the factors responsible for this difference in behavior, it is obvious that these cells are less adapted to stand stress and strain than the fibers.

An interesting observation was made on the structure of the walls of the pitted vessels of the vascular material. Under the phosphoric acid-alcohol treatment the vessel walls which apparently consist of only one layer, separate into fibrils wound at an angle of 45 to 90 degrees to the main axis of the vessel. The pits were found to be parallel to, and in many cases continuous with the limits of the fibrils. (Pl. I, fig. 4.)

#### SUMMARY

The length to width ratio and the disintegration behavior of cells of pulps prepared from parenchymatous and vascular tissues of the mature cornstalk have been observed. These properties of the vascular material are similar to the corresponding properties which have been reported for wood pulps.

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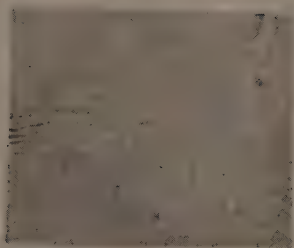
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## PLATE I

## EXPLANATION OF PLATES

- Fig. 1. Separation of delignified cell wall of a fiber from the vascular bundle of a cornstalk into four distinct layers by means of phosphoric acid-absolute alcohol treatment.
- Fig. 2. Transverse swelling of inner layers of cornstalk fiber at places where the outer spiral has been dissolved away or has been pulled away from its normal 90 degree angle.
- Fig. 3. Transverse swelling of inner layers of cornstalk fibers at places where the outside layer has been dissolved.
- Fig. 4. Delignified cell wall of a pitted vessel from the vascular bundle of a cornstalk showing the spirally wound fibrils after phosphoric acid-absolute alcohol treatment.

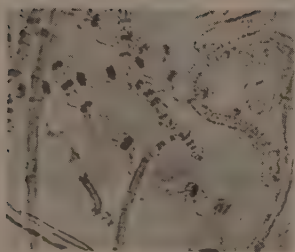
PLATE I



1



2



3



4





# THE LITERATURE OF ALKYLATED CARBOHYDRATES

## IV. FRUCTOSE DERIVATIVES

HAROLD W. COLES

*From the Laboratory of Plant Chemistry, Department of Chemistry, Iowa State College*

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### INTRODUCTION

Much confusion exists in the literature of alkylated fructose, and it has been only recently that the nomenclature and proper numbering of these compounds have been worked out by Haworth, his co-workers, and others, by the use of ingenious degradations. Much of this existing confusion has been because sucrose, one of the earliest sugars investigated with Purdie's reagents and, as has been recently shown, made up of normal glucose and *gamma* or *labile* fructose, on actual hydrolysis reveals normal glucose and *normal* crystalline fructose. Consequently, conclusions as to the structure of the fructose constituent of sucrose and other polysaccharides containing fructose, were wrongly based on the assumption that the fructose was present as the normal form (76).

### MONOMETHYL FRUCTOSE

Monomethyl fructose is mentioned, but not specifically described in 2, 6, 9 and 49. Triacetyl fructose, when treated with methylating agents, forms a *monomethyl triacetyl fructose* (12). Fructose condenses with acetone under different conditions to give two structurally different diacetones. The alpha-diacetone fructose, on methylation, produces *3-monomethyl fructose* (a fructose-pyranose) (3, 16, 21, 29, 30, 43, 46, 59, 74, 75 and 76). This sugar is a normal monomethyl fructose since it gives rise to normal 1, 3, 4, 5-tetramethyl fructose (which see) on complete alkylation. Freudenberg and Doser (29) state that it seems to form a [1, 4]-ring. The melting point of this monomethyl fructose is said to be 115° (30); 122-3° (3) and 128-30° (75). Rotation is shown by the following:  $[\alpha]_D^{20} - 70.5^\circ \rightarrow -53.1^\circ$  (water) (3);  $-84.1^\circ \rightarrow -53.5^\circ$  (water) (75). A *3-methyl gamma-methyl fructoside* can be prepared from the 3-methyl fructose (21, 29, 45). This has a melting point of 143° (45) and a rotation in ethyl alcohol of  $-34.6^\circ$  (45). This fructoside, in turn, on complete methylation forms tetramethyl gamma-methyl fructoside.

The 3-monomethyl fructose is well-characterized by a *3-monomethyl fructosazone* (3, 7, 21, 30, 32, 63, 74, 75 and 76), which is identical with the osazone obtained from the methyl glucose from methyl diacetone glucose (7, 46, 63, 74 and 76). The osazone has been said to have a melting point of 142-4° (3) and 164-5° (6), but the correct melting point is probably 177-9° (30, 74 and 75). The optical rotation is  $[\alpha]_D - 109^\circ \rightarrow -9^\circ$  (alc.) (74) and  $[\alpha]_{HK}$  (yellow)  $-168^\circ (\pm 4^\circ)$  in pyridine (30). *3-Methyl-alpha-diacetone fructose* was not fermented by a selected group of 12 organisms (85).

The second diacetone fructose is  $\beta$ -fructose diacetone. This compound has the free hydroxyl group on carbon one, and consequently *1-methyl fructose* may be obtained. Ohle (43) claimed that the splitting of the isopropylene groups from *methyl- $\beta$ -diacetone fructose* formed a methyl fructose not identical with 3-methyl fructose; which does not give the *6-methyl glucosazone* with phenylhydrazine, and hence must be 1-methyl fructose. Furthermore, it produces, on oxidation with potassium permanganate, an acid,  $C_{12}H_{18}O_7$ , forming a crystalline *potassium salt*. Neither 1-methyl fructose or methyl  $\beta$ -diacetone fructose were digested by a group of organisms (85).

A *6-methyl- $\alpha$ -or  $\beta$ -fructose* is mentioned (5). When 3-monomethyl fructose is oxidized with bromine water, Irvine and Hynd (3) reported that  $\alpha\beta$ -dihydroxy- $\gamma$ -methoxy-butyric acid was obtained, while Anderson and co-workers (75) recovered the monomethyl fructose unchanged, and claim that 3-monomethyl fructose is not oxidized readily.

#### DIMETHYL FRUCTOSE

Dimethyl inulin (levo) is said (18, 19, 23) to form a *dimethyl- $\gamma$ -fructose* which on further methylation leads to tetramethyl  $\gamma$ -methyl fructoside, having a dextro-rotation.

#### TRIMETHYL FRUCTOSE

When trimethyl levan (84) or octamethyl turanose (52) or hydrolyzed, it is said that *1, 3, 4-trimethyl fructose*, the first crystalline gamma sugar isolated, is formed. It forms no *osazone* (52, 84) and may be oxidized to give dimethoxy-hydroxyglutaric acid (52) and also a *dibasic dimethyl lactol acid* which gives a *diethyl ester*, and a crystalline *diamide* (m.p.  $138^\circ$ ) on treatment with dry ammonia (84). The trimethyl fructose forms a tetramethyl fructose on further methylation. This trimethyl fructose has a melting point of  $63^\circ$  (84) and exhibits a rotation of  $[\alpha]_D + 36.9^\circ \rightarrow +34.5^\circ$  (52).

The anhydro-fructose, secured from hydrolysis of the acetyl groups from triacetyl anhydro-fructose, gives *trimethyl anhydro-fructose* (79, 81), a fairly mobile syrup of  $[\alpha]_D + 23.8^\circ$  ( $CHCl_3$ ); b.p.  $166^\circ/0.1$  mm., and  $N_D = 1.4610$ . Distillation induces molecular association (79) to a *di-(trimethyl-anhydrofructose)*.

It will be remembered that fructose produces two structurally different diacetone fructoses. One of these, the fructose  $\alpha$ -diacetone, on partial hydrolysis, gives a fructose  $\alpha$ -monoacetone (levo) (21) from which a *3, 4, 5-trimethyl fructose monoacetone* (levo), a mobile liquid, b.p.  $135.8^\circ/10$  mm., and  $N_D = 1.4575$  may be obtained. On removal of the remaining acetone residue, *3, 4, 5-trimethyl fructose* (levo) a viscous syrup of rotation  $-115.9^\circ$ , is formed.

#### 3, 4, 6-Trimethyl $\gamma$ -Fructose

This trimethyl d-fructose (22, 37, 56, 65, 88) may be obtained from hendecamethyl melezitose (61); from methylated sinistrin (81) or from trimethyl inulin (14, 18, 19, 23, 24, 38, 51, 60, 67, 68, 76, 79, and 82). The physical properties are given in the following table:

B.P.	Pressure	$[\alpha]_D$	Solvent	$N_D$	Ref.
		+30.5°	water	1.4689	(14)
		+22.14°	acetone		(14)
115°	.02	+27.7°	chloroform	1.4675 (14°)	(68)
107°	.05	+23.0°	"	1.4680 (20°)	(71)
110-2°	.02	+25.7°	"	1.4570 (14°)	(81)
115°	.01	+28.2°	"	1.4640 (20°)	(86)

This sugar, a fructofuranose type, reduces permanganate instantly and Fehling's in the cold. It has also been obtained from di-h-fructose anhydride. The sugar forms other compounds readily. These are

I.—3, 4, 6-Trimethyl methyl fructoside, a clear syrup (18, 19, 24, 38) which produces a trimethyl fructose monoacetone (18, 19).

II.—A tetramethyl  $\gamma$ -methyl fructoside (dextro) (23).

III.—A hexamethyl  $<1, 2'> <1', 2>$ -h-difructose anhydride which boils at 150°/0.1 mm., (71),  $N_D = 1.4738$ , non-reducing, easily soluble in alcohol, ether, chloroform and insoluble in water. A rotation at 20° of +31.1° in chloroform is shown.

IV.—A phenylosazone (67, 68, 76, 79, 81, 86), occurring in an anhydrous form, m.p. 137-8° (68) and a hydrated form, of melting point 78-80° (71); 79-80° (81, 86).

The oxidation of this sugar has been thoroughly studied. When treated with nitric acid (67, 68, 76) 3, 4, 6-trimethyl lactol acid ester is first formed. This is a fructo-furonic acid. It is a yellow syrup, reducing Fehling's, of boiling point 132-40° at 0.18 mm.,  $N_D^{15} = 1.4529$  and  $[\alpha]^{16}_D + 27.1^\circ$  (68). This, in turn, can give rise to a tetramethyl lactol acid ester (76) of boiling point 115-30°/0.1 mm.,  $N_D^{15} = 1.4453$ ,  $[\alpha]^{16}_D + 2^\circ$  (water), characterized by a tetramethyl amide of m.p. 100-1°. Or the above-mentioned lactol acid ester may be further degraded to form d-2, 3, 5-trimethyl  $\gamma$ -arabonolactone, m.p. 31-2, and this still further to 1-dimethyl tartaric acid.

#### TETRAMETHYL FRUCTOSE

Tetramethyl fructose is mentioned in 9, 11, 15 and 17, and tetramethyl  $\gamma$ -fructoses are discussed in 10, 28, 31, 33, 36, 41, 44, 46, 48, 55 and 60. Figures on the mutarotation of the tetramethyl derivative is given (39) and it is stated that attempts (4) to prepare the anilide of tetramethyl fructose resulted in a syrup.

#### 1, 3, 4, 6-Tetramethyl $\gamma$ -Fructose

Under this sugar will also be included the tetramethyl fructose described erroneously as having the methyl groups on carbons 1, 4, 5 and 6. This tetramethyl fructose of the labile or  $\gamma$ -form (fructofuranose) represents the fructose constituent of sucrose, and is secured when heptamethyl or octamethyl sucrose are hydrolyzed (8, 13, 14, 24, 25, 26, 34, 35, 38, 40, 59, 69, 76 and 78). It also results from the methylation of trimethyl  $\gamma$ -fructose (57, 61); from the sucrose residue of methylated raffinose (32, 33, 64); and is said to result from the mixture of methylated fructosides from fructose (12).

The physical properties of this important sugar are found in the following table:

B.P.	Pressure	N <sub>D</sub>	[α] <sub>D</sub>	Solvent	Ref.
154°	13.00	1.4545	+14.04°	alcohol	(14)
			—31.7°	initial-water	(14, 25, 76)
110-2°	0.35		31.7°	initial-water	(40)
148.5°	10.00	1.4554	32.9°	initial-water	(25)
			32.6°	water	(44)
		1.455	32.26°	water	(51)
		1.4513	31.3°	water	(59)
			34.1°	3 days-water	(78)

It is a colorless syrup (12, 13, 51), unstable toward potassium permanganate (13, 14, 59), and is acted on by acids and alkalis. The action of N/30 alkali results in changes which cause a positive test with Schiff's reagent. Even with neutral hypiodite, purest samples gave a figure corresponding to a content of 2.8 per cent of tetramethyl glucose (59).

The action, on oxidation with nitric acid, of the tetramethyl fructose is characteristic: a 3, 4, 6-trimethyl fructuronic (lactol) acid is first formed, which is a liquid reducing Fehling's but losing this property when the reducing group is protected by a methyl residue (53, 76). This *tetramethyl fructuronic acid* forms an *ethyl ester*, stable toward alkali, which is identified by its crystalline *amide*, m.p. 99-100°.

The 3, 4, 6-trimethyl lactol acid also forms an *ethyl ester*, which undergoes profound decomposition in the presence of hot alkali. The lactol acid may be further degraded as shown:

(1) By potassium permanganate, in the presence of dilute sulfuric acid, to 2, 3, 5-trimethyl d-arabonolactone to l-dimethyl tartaric acid.

(2) By alkaline potassium permanganate (53). An unsaturated acid is first obtained which breaks down to 2, 4-dimethyl d-erythronic acid, identified by its crystalline *amide* (m.p. 104-5°) and from the methyl trimethoxy butyrate also obtained, recognized by its crystalline *amide*, m.p. 58-9°.

A semi-lactide of trimethyl tetrahydroxyvaleric acid is reported (26) from the oxidation of this sugar and also xylotrimethoxyglutaric acid identified by its crystalline *amide*, m.p. 101°, [α]<sub>D</sub> —76° (water) (59).

This tetramethyl γ-fructose is apparently the carbohydrate constituent present in hexose diphosphoric acid. Thus, it is reported that a β-methyl hexoside is secured from the hexosediphosphoric acid which, on methylation, forms a *tetramethyl β-methyl hexoside*, [α]<sub>5461</sub><sup>20</sup> —6.8°, and on removal of the glycoside group, an αβ-tetramethyl hexose, [α]<sub>Hg</sub><sup>20</sup> (green) +30°, is obtained (54, 62, 70). This rotation is very close to that given by Irvine, Haworth and others for αβ-tetramethyl γ-fructose. A *tetramethyl ester of trimethyl-hexose-diphosphoric acid* is reported (83).

A *tetramethyl -methyl fructoside (dextro)* is obtained from dimethyl γ-fructose (23). It has N<sub>D</sub> = 1.4468 and b.p. 95-105°/0.15 mm., and exhibits a rotation in alcohol of +44.9° (25).

### 1, 3, 4, 5-Tetramethyl Fructose

This sugar is the normal, or pyranose, form and is obtained on hydrolysis of the *tetramethyl β-methyl fructoside* secured on methylation of the normal β-methyl fructoside (—172°) from normal d-fructose (—130°) (1, 12, 13, 14, 21, 22, 57, 76). It is also obtainable from the corresponding



methylated  $\alpha$ -methyl fructoside (72), and furthermore is said to result from the breaking down of methylated inulin (23, 38). It also may be obtained from the corresponding tetramethyl  $\beta$ -fructose (48). The tetramethyl fructose in the syrup form (37, 42, 60, 67, 80) distills without decomposition (1) and is said (20) to crystallize in two different forms, having different melting points, square plates, m.p.  $98.9^\circ$  (22) and pointed prisms, m.p.  $92^\circ$  (22). The latter crystals are not believed to be the  $\beta$ -form. The sugar was first isolated by Purdie and Paul (1) and was said to have the methoxyl groups attached to carbons 1, 3, 4 and 6 (22), but this opinion was later shown to be incorrect (57).

Physical constants are:

M.P.	$[\alpha]_D$	Solvent	Ref.
	$-20.9^\circ$	Water	(14)
	$-20.2^\circ$	Alcohol	(14)
$98.9^\circ$	$-121^\circ$	Water	(8, 13)
$95.7^\circ$	$-87.3^\circ$	Water	(21)
$98.9^\circ$	$-87.7^\circ$	Alcohol—3 days	(22)
$98.9^\circ$			(57)
$98.9^\circ$	$-84.7^\circ$	Benzene—7 days	(22)
$92^\circ$	$-85.7^\circ$	Water	(22)
$97^\circ$	$-119^\circ$	"	(72)
$92.3^\circ$			(73)
$98.9^\circ$	$-124^\circ$	"	(75)
	$-123^\circ$	"	(76)

The 1, 3, 4, 5-tetramethyl  $\beta$ -methyl fructoside obtained from  $\beta$ -methyl fructoside (22, 76) or from trimethyl fructose (levo) (21) is a colorless liquid of b.p.  $130.4^\circ/11$  mm.,  $105.6^\circ/0.06$  mm. (57), distilling without decomposition (1), of  $N_D^{16} \pm 1.4560$  (57) finally crystallizing to hard flat prisms of melting point  $33.4^\circ$ .  $[\alpha]_D^{17} -149.1^\circ$  (water) (57), without the power to reduce Fehling's. The corresponding 1, 3, 4, 5-tetramethyl  $\alpha$ -methyl fructoside (72, 87) b.p.  $75.80^\circ/0.1$  mm.,  $N_D = 1.4637$ ,  $[\alpha]_D + 85^\circ$  (72);  $+83.7^\circ$  (chloroform) (87);  $+16.7^\circ$  (water) may be secured by the methylation of  $\alpha$ -methyl fructoside.

The oxidation (50) of the 1, 3, 4, 5-tetramethyl fructose [2, 6] has provoked much discussion. The following products have been described as resulting from different methods of oxidation.

(a) *Trimethyl lactol ethyl fructuronate* (57, 76).

Has a melting point of  $87.8^\circ$ ,  $[\alpha]_D -98^\circ$  in water. Reduces Fehling's, and is unstable in hot alkali, giving a yellow solution. This compound was wrongly believed (22) to be a diethyl ester of dimethoxy-glutaric acid.

(b) d-Arabotrimethoxy-glutaric acid (46, 57).

Secured by degradation of (a). Characterized by an amide, m.p.  $227.8^\circ$  (decomp'n),  $[\alpha]_D = -48^\circ$  (water).

(c) Inactive dimethoxy succinic acid (46, 57).

Occurs along with (b).

(d) *Trimethyl lactol fructuronic acid* (47, 57, 66, 68, 76, 77).

This may be obtained from (a) or directly from the tetramethyl fructose (47, 66, 68, 76, 77). The boiling point is  $119.20^\circ$  (47, 57) and in

methyl alcohol the rotation is  $-94^\circ$  (57). It is declared to be strongly reducing. A potassium salt has been described (34).

(e) *Tetramethyl lactol methyl fructuronate* (47, 57, 75).

May be prepared from (d). Consists of colorless, hexagonal plates, stable to alkali, unchanged on boiling with HCl (47) and Fehling's is not reduced. The melting point is said to be  $102.3^\circ$  (47, 75), with rotations as follows:  $[\alpha]_D^{20} -116^\circ$  (methyl alcohol);  $-129^\circ$  (water) and  $[\alpha]_D^{22} -130^\circ$  (water) (75).

(f) *Tetramethyl lactol fructuronamide* (57, 75).

A derivative of (e). Needles or long, flat, thin plates (57), m.p.  $118-9^\circ$ .  $[\alpha]_D -137^\circ$  (water) (approx.).

(g) d-2, 3, 4-Trimethyl  $\delta$ -Arabonolactone (47, 58, 66, 68, 76, 77).

The degradation product of (d), which may, however, be secured directly from the tetramethyl fructose (75, 76). The melting point is  $31.2^\circ$  (68);  $33^\circ$  (58) and in water the initial rotation is  $+44.5^\circ$ , changing to  $+25.5^\circ$  in 20 days (58). This compound may be identified as (b) or (c) (76).

(h) Dimethyl mesotartaric acid (46).

Recognizable by a crystalline amide.

(i) Dimethoxybutyric acid lactone (22, 35, 40).

Formed from the corresponding acid lactone by assuming the oxidation, by permanganate, of the enol form of 1, 3, 4, 5-tetramethyl fructose.

(j) Trimethoxy valerolactone (34, 35, 40, 51).

Oxamide also mentioned. The addition of water forms trimethoxy valerianic acid. On further oxidation of this acid, (k) is produced.

(k) Trimethoxy glutaric acid (27).

Anhydride described.

When 1, 3, 4, 5-tetramethyl fructose is reduced with sodium amalgam (35), *tetramethyl hexitol* (probably derivatives of sorbitol and mannitol) is formed, exhibiting a specific rotation, in water, of  $+10.8^\circ$ . The tetramethyl fructose is not fermented by a selected group of twelve organisms (85).

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# EFFECT OF SALTS ON ALKALI DISINFECTION<sup>1</sup>

O. E. LOWMAN WITH J. H. BUCHANAN AND MAX LEVINE

*From the Departments of Chemistry and Bacteriology, Iowa State College*

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The mechanical washing of bottles is a universally adopted practice in the beverage, dairy, and other similar industries. The modern bottle washing machines combine the process of cleansing and sterilizing in a single operation. These machines use commercial washing compounds as detergents and germicides. Sodium hydroxide is used as a base to which has been added some milder alkali such as sodium carbonate or tri-sodium phosphate.

There is considerable disagreement concerning the theory of disinfection by alkalis. The general conception seems to be that the free hydroxyl-ion concentration is the important factor (7, 8, 10, 11, 13). Some investigators have pointed out that certain physical properties of the solution such as buffer index, osmotic pressure, and surface tension are significant. Others claim that adsorption, swelling, and other properties inherent in the bacteria have an important bearing on the germicidal efficiency of the alkalis (9, 12).

In 1926 the chemical and bacteriological laboratories at Iowa State College became interested in the relative germicidal efficiency of sodium hydroxide and various commercial washing powders (2, 3, 4, 5, 6). From data secured the workers were led to believe that the concentration of the undissociated molecule of sodium hydroxide, as well as the concentration of the hydroxyl-ion, was an important factor in alkali disinfection. With this in mind the present investigation was undertaken to present a series of data from which it was hoped an adequate theory of the chemistry of alkali disinfection might be offered.

## TEST ORGANISM AND TECHNIQUE

For this work it was thought best to use a spore-forming organism with a rather high resistance to alkalis and temperature. This organism and methods of preparing the culture have been fully described in another paper (2). It was originally isolated from a sample of spoiled ginger ale. It has been described as a "Gram positive rod (about  $1.0\mu$  by  $2.0$  to  $4.0\mu$ ), facultative, motile, with central spores equal to or slightly less than the diameter of the cells. The vegetative cells occurred singly, in pairs, and occasionally in short chains. Gelatin was liquefied, milk slowly curdled (rennet) and peptonized, nitrates were reduced to nitrites but not gas, and indol was formed. Acid was formed from glucose, but not from lactose or sucrose, and starch was hydrolyzed. Colonies on agar were strikingly similar to those of *B. subtilis*."

<sup>1</sup>From a thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Iowa State College.

This organism was well adapted to plate cultures since it grew well on nutrient agar, forming distinct colonies in about two days with little tendency to confluence. When the organism was transferred to agar from solutions just alkaline to methyl orange, its growth was not inhibited in any manner.

A technique in the disinfection tests had to be developed which would conform in a general way to the conditions found in washing machines and not introduce too great an error in bacterial counts. The method has been fully described in previous papers (2, 4), and only brief mention of it will be made here.

The bacterial suspension for the tests was made by adding a weighed amount (about 0.02 grams) of spore-sugar mixture to 10 cc. of sterile tap water. After thorough shaking, the suspension was filtered through a sterile filter paper of fine grade, and the filtrate constituted the bacterial suspension for the tests.

The test alkali solution (100 cc.) was placed in a 200 cc. round bottom Woulff flask provided with three necks. A glass stirrer was fitted through a stopper in the middle neck, the other two openings were stoppered with cotton and the whole sterilized in an autoclave at 15 pounds for 15 minutes. After cooling, the flask was placed in a De Khotinsky water bath which had been brought to the proper temperature, and the stirrer set in motion. Care was taken that no bubbles or foaming developed.

When the test solution had attained and remained at the desired temperature, one cc. of the bacterial suspension was carefully introduced under the surface of the test solution by means of a calibrated capillary pipette. At desired time intervals, five cc. portions were removed to 150 cc. sterilized Erlenmeyer flasks containing 45 cc. of sulfuric acid (with methyl orange indicator) of sufficient strength to just neutralize the five cc. of test alkali solution. This operation stopped simultaneously the effects of alkali and temperature. One cc. portions were plated on nutrient agar (Difco), incubated at 27°C. for 48 hours, and the number of surviving bacteria determined. It was intended to employ a suspension giving an initial count of about 1,000,000 per unit volume (5 cc.).

The sodium hydroxide used for the test solutions was prepared by making a saturated solution from C. P. Merck stick sodium hydroxide. After standing, the saturated solution was decanted and filtered through glass wool to remove any carbonate which had accumulated. This was made up to one normal by titration against a standard acid (phenolphthalein indicator) and kept in stoppered Pyrex flasks as a stock solution. The hydroxide solutions used in the germicidal tests were made up as needed from this solution. When sodium halides were used in the tests, the dry C. P. Merck salt was weighed and added to the hydroxide test solution before sterilization. The only variation in the technique was in the case of sodium iodide added to the alkali test solutions. Employing the usual technique with this disinfecting mixture, it was noted in a preliminary test that the killing time was greatly reduced below what had been anticipated. Investigation revealed that free iodine had been liberated (starch test). This difficulty was overcome by making the five cc. portions of alkali which had been removed from the disinfection flasks, just acid to phenolphthalein instead of just alkaline to methyl orange as in the usual technique. After this variation was introduced, no free iodine was detected (starch test).

## EXPERIMENTAL

## I. GERMICIDAL EFFECT OF DISTILLED WATER AND CERTAIN SODIUM HALIDE SOLUTIONS AT 60°C.

It was thought that it would be of interest to know the effect of temperature and of certain sodium halide solutions on the test organism at 60°C. To secure the effect of temperature on the organism, one cc. of the bacterial suspension was introduced into the disinfection flasks containing 100 cc. of distilled water and the whole maintained at the desired temperature (60°C.) in the De Khotinsky bath. Five cc. portions were removed at different time intervals and cooled in 45 cc. of distilled water. Bacterial counts were made by plating one cc. portions of various dilutions on nutrient agar. Since some previous work had been done to determine the effect of two per cent (0.342M) sodium chloride solution on the test organism, the same concentrations (0.342M) of sodium iodide and sodium fluoride were selected in order to make the work comparable. Sodium bromide was not used in these tests because it was anticipated that the sodium iodide solutions would be at least as effective. In the sodium iodide tests it was found necessary to add a few drops of 0.25M sodium hydroxide in order to prevent the liberation of free iodine.

The bacterial counts of the survivors at different time intervals are given in table 1. The logarithms of the percentage of survivors are plotted against time in minutes in figure 1. Curve A is a survivor's curve which represents a large number of tests with 0.25M sodium hydroxide at 60°C. and is used in this figure for comparison.

TABLE 1. *Germicidal effect of distilled water, 0.342M NaI, and 0.342M NaF at 60°C.*

Time in Min.	Surviving bacteria in 5.0 cc.			Percentage surviving bacteria			Log. percentage survivors		
	Dis- tilled water	NaI	NaF	Dis- tilled water	NaI	NaF	Dis tilled water	NaI	NaF
0	660,000	660,000	745,000	100.0	100.0	100.0	2.000	2.000	2.000
10	460,000	455,000	439,000	69.7	69.0	59.0	1.84323	1.83885	1.77085
15	390,000	320,000	419,000	59.1	48.5	56.2	1.77159	1.68574	1.74974
20	615,000	430,000	407,000	93.3	65.1	54.7	1.96988	1.81358	1.73799
25	605,000	-----	400,000	91.7	-----	53.7	1.96237	-----	1.72997
30	500,000	315,000	242,000	75.9	47.7	32.5	1.88024	1.67897	1.51188
35	435,000	355,000	271,000	65.9	53.8	36.4	1.81889	1.73078	1.56110
40	-----	300,000	235,000	-----	45.0	31.6	-----	1.65369	1.49969
45	475,000	385,000	111,000	72.0	58.4	14.9	1.85733	1.76641	1.17319
50	920,000	225,000	219,500	113.94	34.1	29.4	2.05652	1.53275	1.46835

In figure 1, which was made from data compiled in table 1, it is interesting to note that curves B, C, and D all show a decided reduction in bacterial count during the first 15 minutes of exposure. This phenomenon was found to be common in all our disinfection tests and would seem to indicate that the weaker bacteria were unable to withstand (for a greater length of time) the effects of temperature and the halides of the test solu-

tions. The increased bacterial count directly following this period may be caused by the breaking up of minute clumps of the spore suspension which passed through the filter paper. The general trend of the curves B, C, and D indicate that a temperature of 60°C. and that 0.342M sodium iodide and sodium fluoride do have a germicidal effect on the test organism, reducing the bacterial counts approximately 18, 38, and 75 per cent, respectively (in 50 minutes). Sodium fluoride is more toxic than sodium iodide at 60°C. This may be due to the greater hydrolytic effect of the fluoride causing the test solution to become more alkaline. Curve A is used in this figure for comparison. It was plotted from the average data compiled from a large number of tests made with 0.25M sodium hydroxide at 60°C. and shows the lethal effect of this concentration of alkali on the test organism at this temperature.

## II. EFFECT OF THE ADDITION OF SODIUM HALIDES ON THE GERMICIDAL EFFICIENCY OF 0.25M SODIUM HYDROXIDE AT 60°C.

From some previous work published from these laboratories, it was known that the addition of a neutral salt (sodium chloride, two per cent or

TABLE 2. *Effect of added salts on germicidal efficiency of 0.25M sodium hydroxide at 60°C.*

### Series A

Dates	8-20-27	2-11-28				
Time in minutes	Surviving bacteria in 5.0 cc.		Percentage Survivors		Log. percentage Survivors	
Observations with 0.25M NaOH						
0	933,000	60,000	100.00	100.00	2.000	2.000
5	.....	.....	.....	.....	.....	.....
10	586,000	27,000	62.9	44.80	1.799	1.651
15	510,000	12,000	54.7	20.70	1.734	1.316
20	318,000	5,300	33.9	8.75	1.531	0.942
25	123,000	1,400	13.2	2.33	1.122	0.367
30	58,000	650	6.16	1.08	0.791	0.033
35	14,000	250	1.52	0.42	0.193	1.623
40	2,400	.....	.257	.....	1.411	.....
45	750	.....	.081	.....	2.909	.....
Observations with 0.25M NaOH + 0.342M NaCl						
0	933,000	60,000	100.00	100.00	2.000	2.000
5	.....	40,000	.....	66.60	.....	1.823
10	210,000	10,000	22.50	17.20	1.353	1.236
15	71,000	2,000	7.61	3.34	0.882	0.524
20	7,800	270	0.842	0.45	1.926	1.653
25	280	.....	0.030	.....	2.478	.....



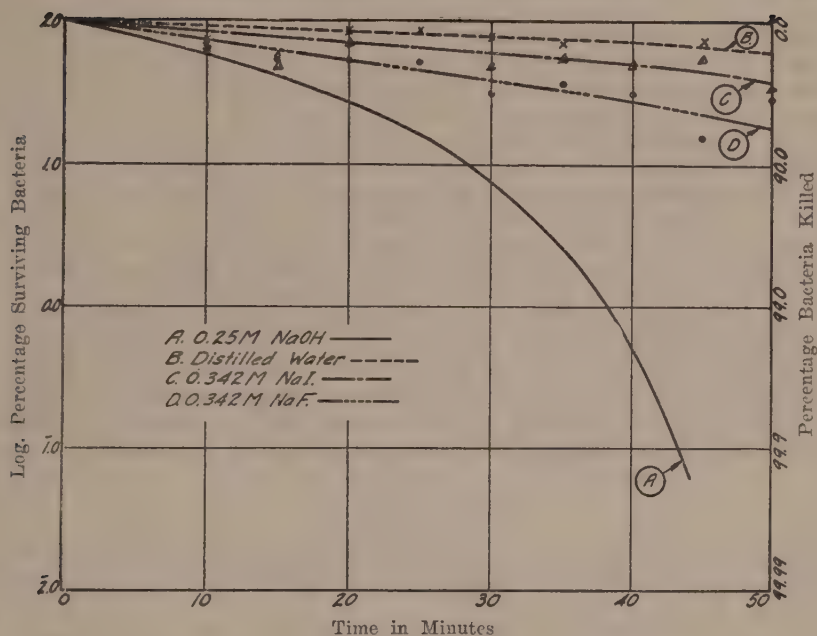


Fig. 1. Germicidal efficiency of distilled water and 0.342M sodium halides compared with 0.25M NaOH at 60°C.

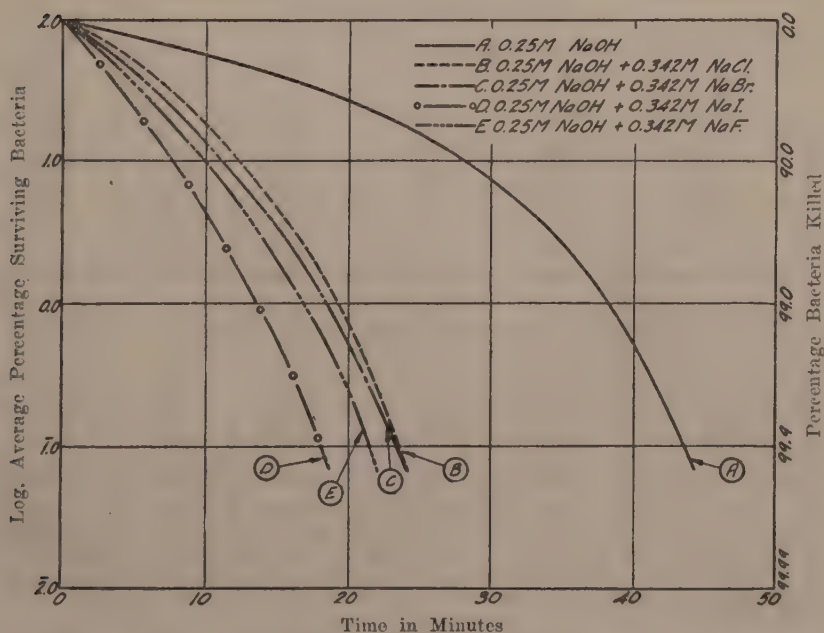


Fig. 2. Effect of sodium halides on germicidal efficiency of NaOH at 60°C.

0.342M) to 0.25M sodium hydroxide greatly increased the germicidal efficiency of the alkali at 60°C. (5, 6). It was thought that it would be of interest to present data showing the comparative effect of other sodium halides (sodium bromide, sodium iodide, and sodium fluoride) with that of the chloride-hydroxide mixture of the same concentration and at the same temperature. These tests were run in duplicate according to the previously described technique. The proper amount of the dry halide (C. P. Merck) was carefully weighed and added to the alkali in the disinfection flasks and then the whole sterilized. A control test of 0.25M sodium hydroxide was run with each individual test. The results are recorded in table 2, series A, B, C, and D. The logarithm of the average percentage of the survivors of the individual tests is plotted against time in figure 2.

Table 2, series A, B, C, and D, shows the increased germicidal effect of the 0.25M sodium hydroxide at 60°C. when the various sodium halides are added in 0.342M concentration. In figure 2, the average results are plotted against time in minutes. Curve A represents an average of all controls of 0.25M sodium hydroxide alone run with the tests of the various

TABLE 2. (Continued)

## Series B

Dates	4-7-28	4-14-28				
Time in minutes	Surviving bacteria in 5.0 cc.		Percentage survivors		Log. percentage survivors	
Observations with 0.25M NaOH						
0	720,000	1,050,000	100.00	100.00	2.000	2.000
5						
10	195,000	268,000	26.95	25.5	1.431	1.406
15	170,000	179,000	23.60	17.0	1.373	1.230
20	116,000	163,000	16.05	15.47	1.205	1.189
25	83,000	81,000	11.45	7.66	1.059	0.884
30	51,000	57,000	7.02	5.45	0.846	0.736
35	20,000	12,000	2.82	1.095	0.450	0.039
40	6,400	2,800	0.89	0.267	1.944	1.426
45	850	250	0.114	0.024	1.057	2.376
Observations with 0.25M NaOH + 0.342M NaBr						
0	720,000	1,050,000	100.00	100.00	2.000	2.000
5	188,000	214,000	26.10	20.40	1.417	1.309
10	67,000	33,000	9.24	3.095	0.966	0.491
15	44,000	22,000	6.09	2.065	0.785	0.315
20	6,000	4,600	0.835	0.438	1.922	1.641
25	400	550	0.056	0.053	2.745	2.724

TABLE 2. (Continued)

## Series C

Dates	6-13-28	6-16-28				
Time in minutes	Surviving bacteria in 5.0 cc.		Percentage survivors		Log. percentage survivors	
Observations with 0.25M NaOH						
0	4,000,000	390,000	100.00	100.00	2.000	2.000
10	1,132,000	123,000	28.35	31.60	1.453	1.499
15	1,072,000	78,000	26.90	19.90	1.431	1.299
20	726,000	37,000	18.15	9.50	1.259	0.978
25	197,000	16,000	4.93	4.20	0.693	0.623
30	-----	5,600	-----	1.44	-----	0.158
35	83,000	1,200	2.07	0.295	0.316	1.469
40	20,000	-----	0.505	-----	1.703	-----
45	2,600	-----	0.066	-----	2.823	-----
Observations with 0.25M NaOH + 0.342M NaI						
0	4,000,000	390,000	100.00	100.00	2.000	2.000
2	675,000	-----	16.85	-----	1.227	-----
4	593,000	-----	14.80	-----	1.170	-----
5	-----	58,000	-----	14.90	-----	1.173
8	188,000	-----	4.70	-----	0.672	-----
10	225,000	4,500	5.60	1.15	0.748	0.061
12	-----	-----	-----	-----	-----	-----
14	37,000	-----	0.925	-----	1.971	-----
15	-----	800	-----	0.205	-----	1.312
16	16,000	-----	0.390	-----	1.561	-----
18	4,500	-----	0.114	-----	1.057	-----

halide-hydroxide mixtures. This curve indicates an average killing time of 43.5 minutes for 99.9 per cent of the test organisms. Curves B, C, D, and E show a distinct decrease in the killing time when various halides are added to the alkali test solution. The sodium chloride and bromide-hydroxide mixtures (curves B and C) were approximately the same (23.25 minutes), the fluoride-hydroxide mixture (curve E) occupying an intermediate position (21 minutes), with the iodide-hydroxide mixture (curve D) most effective of all (18 minutes). Thus, with the addition of 0.342M concentration of the salts, a decrease of 46.5, 46.5, 58.6, and 51.7 per cent, respectively, was obtained in the time to effect a reduction of 99.9 per cent of the exposed bacteria. The slightly increased effectiveness of the fluoride mixture over the chloride and bromide mixtures may be due to the hydrolyzing effect of the salt. The pH of this mixture was slightly higher as will be shown in a later table. By titration against a standard acid, this mixture gave a greater alkalinity by 0.04N than the control test solution

TABLE 2. (Continued)

## Series D

Dates	7-10-28	7-11-28				
Time in minutes	Surviving bacteria in 5.0 cc.		Percentage survivors		Log. percentage survivors	
Observations with 0.25M NaOH						
0	780,000	281,000	100.00	100.00	2.000	2.000
10	318,000	164,000	40.60	58.20	1.609	1.765
15	245,000	-----	31.35	-----	1.496	-----
20	159,000	73,000	20.30	25.90	1.308	1.413
25	79,000	55,000	10.05	19.65	1.002	1.293
30	25,000	28,000	3.17	10.00	0.501	1.000
35	4,500	13,000	0.58	4.47	1.763	0.650
40	900	2,400	0.115	0.855	1.061	1.932
45	-----	500	-----	0.178	-----	1.250
Observations with 0.25M NaOH + 0.342M NaF						
0	780,000	280,000	100.00	100.00	2.000	2.000
5	184,000	-----	23.60	-----	1.373	-----
6	-----	78,000	-----	27.80	-----	1.444
8	-----	47,000	-----	16.70	-----	1.223
10	73,000	31,000	9.35	11.10	0.971	1.045
12	-----	13,000	-----	4.63	-----	0.666
14	-----	7,200	-----	2.54	-----	0.405
15	17,000	-----	2.24	-----	0.350	-----
16	-----	4,500	-----	1.58	-----	0.199
20	700	800	0.09	0.285	2.954	1.455

(0.25M sodium hydroxide). Later in this paper it will be shown that when this factor is taken into consideration, the fluoride-hydroxide mixture falls in the same class with the chloride and bromide mixtures. The iodide-hydroxide is considerably more effective and, while no satisfactory explanation is at hand, several possibilities will be offered later.

### III. EFFECT OF PRELIMINARY SOAKING ON BACTERIAL SPORES IN VARIOUS SODIUM HALIDE SOLUTIONS BEFORE DISINFECTION TESTS

It was thought it would be of some value to know the effect of a preliminary soaking on the bacterial spores before disinfection. It is common practice to soften and remove dried albuminous material from pipettes by soaking in a saturated sodium chloride solution and, also, one worker had observed that sodium chloride decreased the solubility of protein and at the same time increased the lowering of the surface tension (1). Experiments were arranged whereby the spores were soaked for one hour in a 0.342M halide solution before inoculations into the 0.25M sodium hydroxide test solution. In the case of sodium iodide, it was necessary to add a few drops of alkali to prevent the liberation of free iodine. In each instance



TABLE 3. *Effect of preliminary soaking of bacterial spores in various halide solutions on germicidal efficiency of NaOH at 60°C.*

Series A						
NaCl						
Dates	8-20-27	2-11-28				
Preliminary treatment	None					
Time in minutes	Surviving bacteria in 5.0 cc.	Percentage survivors		Av. Petg. survivors	Log. Av. Petg. survivors	
Observations with 0.25M NaOH + 1 cc. 0.342M NaCl solution						
0	933,000	60,000	100.00	100.00	100.00	2.000
10	491,000	48,000	52.70	80.00	66.35	1.822
15	430,000	13,000	46.60	22.20	34.40	1.537
20	258,000	4,000	27.65	6.67	17.16	1.235
25	126,000	-----	13.50	-----	13.50	1.130
30	48,000	480	5.12	0.79	2.96	0.471
35	12,000	-----	1.36	-----	1.36	0.134
40	3,700	-----	0.396	-----	0.396	1.598
45	-----	-----	-----	-----	-----	-----
Observations with 0.25M NaOH						
Preliminary treatment	Soaked 1 hour in 0.342 M NaCl					
0	1,440,000	54,000	100.00	100.00	100.00	2.000
10	1,115,000	29,000	77.10	53.75	65.43	1.816
15	790,000	13,000	54.80	24.20	39.50	1.597
20	541,000	8,000	37.60	15.90	26.75	1.427
25	321,000	2,200	22.15	3.90	13.03	1.115
30	75,000	800	5.25	1.30	3.78	0.577
35	18,000	100	1.27	0.185	0.727	1.862
40	3,500	-----	0.24	-----	0.24	1.380
45	-----	-----	-----	-----	-----	-----

a control of 0.25M sodium hydroxide was inoculated with a spore suspension which had not been subjected to preliminary soaking. In order to make the salt effect comparable in the two tests, one cc. of the 0.342M halide solution used was added to the control tests, respectively, since when one cc. of the soaked suspension was inoculated into the test solutions, approximately one cc. of the salt solution was added with it. Duplicates were run in each instance.

TABLE 3. (Continued)

Series B

NaBr

Date	4-14-28	4-7-28				
Preliminary treatment	None					
Time in minutes	Surviving bacteria in 5.0 cc.		Percentage survivors		Av. Petg. survivors	Log. av. Petg. survivors
Observations with 0.25M NaOH + 1 cc 0.342M NaBr solution						
0	1,050,000	720,000	100.00	100.00	100.00	2.000
10	622,000	246,000	59.20	34.20	46.70	1.669
15	446,000	216,000	42.50	30.00	36.25	1.559
20	332,000	115,000	31.50	15.90	23.70	1.375
25	245,000	88,000	23.40	12.15	17.77	1.249
30	129,000	54,000	12.30	7.45	9.87	0.994
35	55,000	25,000	5.24	3.50	4.37	0.640
40	4,100	4,600	0.39	0.64	0.515	1.712
45	-----	-----	-----	-----	-----	-----
Observations with 0.25M NaOH						
Preliminary treatment	Soaked 1 hour in 0.342 NaBr					
0	1,800,000	675,000	100.00	100.00	100.00	2.000
10	459,000	222,000	25.50	32.80	29.15	1.465
15	335,000	176,000	18.60	26.00	22.30	1.348
20	150,000	112,000	8.32	16.55	12.43	1.094
25	107,000	66,000	5.95	9.78	7.86	0.895
30	36,000	25,000	1.995	3.69	2.84	0.453
35	9,000	14,000	0.505	2.14	1.32	0.121
40	350	1,800	0.020	0.259	0.139	1.143
45	-----	-----	-----	-----	-----	-----

Experiments with sodium fluoride were not made because the hydrolytic effect of the salt showed an alkalinity of 0.04 N by titration with a standard acid (methyl orange indicator).

The data obtained in these experiments are compiled in table 3, series A, B, and C, showing the effect of soaking in sodium chloride, sodium bromide, and sodium iodide solutions, respectively. Different amounts of sample of the spore-sugar mixture were used in these tests, hence the variations in the initial counts. In figure 3, the logarithm of the average present survivors of the duplicate tests is plotted against time in minutes.

TABLE 3. (Continued)

Series C

NaI

Dates	4-21-28	5-5-28				
Preliminary treatment	None					
Time in minutes	Surviving bacteria in 5.0 cc.		Percentage survivors		Av. Petg. survivors	Log. av. Petg. survivors
Observations with 0.25M NaOH + 1 cc. of 0.342M NaI solution						
0	395,000	1,262,000	100.00	100.00	100.00	2.000
10	93,000	241,000	23.50	19.00	21.25	1.327
15	36,000	153,300	9.10	12.10	10.06	1.003
20	18,000	20,000	4.63	1.54	3.09	0.490
25	5,000	-----	1.27	-----	1.27	0.104
30	2,300	5,500	0.57	0.435	0.503	1.702
35	250	2,200	0.064	0.174	0.119	1.766
40	-----	-----	-----	-----	-----	-----
45	-----	-----	-----	-----	-----	-----
Observations with 0.25M NaOH						
Preliminary treatment	Soaked 1 hour in 0.342M NaI					
0	455,000	300,000	100.00	100.00	100.00	2.000
10	138,000	180,000	30.40	60.00	45.20	1.655
15	91,000	157,000	20.00	52.50	36.25	1.559
20	32,000	56,000	7.05	18.65	12.85	1.109
25	13,000	32,000	2.75	10.07	6.41	0.807
30	1,800	4,000	0.385	1.42	0.903	1.956
35	380	500	0.083	0.166	0.124	1.093
40	-----	-----	-----	-----	-----	-----
45	-----	-----	-----	-----	-----	-----

Control 0.25M sodium hydroxide solutions represented by curves A, B, and C in figure 3 had one cc. of the respective halide solutions added before inoculation with the non-soaked spore suspension. This made the salt effect comparable with the tests represented by curves A', B', and C', in which approximately one cc. of the respective halide solutions were added when the soaked spore suspensions were inoculated into the test solutions. Within the limits of experimental errors, practically no effect is

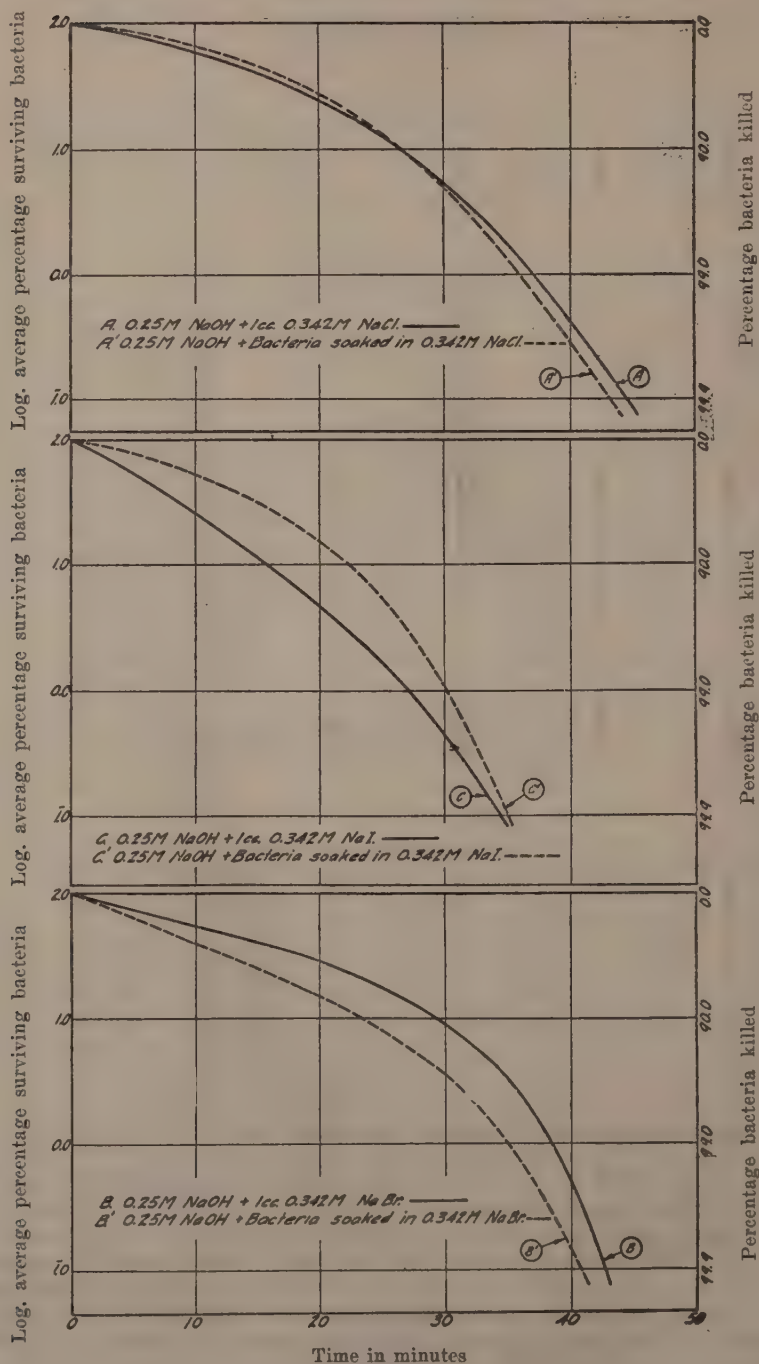


Fig. 3. Effect of preliminary soaking in sodium halide solutions on bacteria before disinfection with NaOH at  $0^{\circ}\text{C}$ .



noticeable because of the presoaking of the test organism. Where sodium iodide was employed (curves C and C') the disinfection time is noticeably less beyond probable experimental error, but the one soaking-effect is not the important factor.

#### IV. pH VALUES OF TEST SOLUTIONS (BEFORE AND AFTER STERILIZATION) AND COMPARATIVE DISINFECTION TIMES

It is of interest to compare the pH values of the various test solutions with their respective killing times since some investigators in this field have maintained that the germicidal efficiency of an alkali may be measured by its pH value (7, 11). Also, it was thought it would be of interest to know the effect of sterilization on the pH of the test solutions. The pH determinations on the different solutions were made at 25°C., both before and after sterilization, using the usual hydrogen platinum electrode and a Leeds and Northrup student's potentiometer (new type) with a portable d'Arsonval galvanometer. These solutions were sterilized in an autoclave at 15 pounds for 15 minutes. The results of these determinations, together with the respective killing times, are recorded in table 4. It is evident that the greatly increased germicidal efficiency of the halide-hydroxide mixture is not caused by a change in pH.

TABLE 4. *pH values of test solutions compared with their respective disinfection times*

Test solution	pH (25°C.) (before sterilization)	pH (25°C.) (after sterilization)	Disinfection time (in minutes at 60°C)
0.25M NaOH	12.86	12.86	43.5
0.25M NaOH + 0.342M NaCl	12.88	12.88	23.25
0.25M NaOH + 0.342M NaBr	12.85	12.84	23.25
0.25M NaOH + 0.342M NaI	12.86	12.83	18.00
0.25M NaOH + 0.342M NaF	12.91	12.92	21.00
0.342M NaF	6.68	6.70	(Reduced bacterial count about 75% in 50 min. at 60°C.)

#### V. GENERAL DISCUSSION AND THEORETICAL CONSIDERATIONS

In the beginning it was pointed out that there was no general agreement among investigators as to type of action involved in disinfection by alkalis. The consensus of opinion seemed to be that it was the concentration of the OH<sup>-</sup> ion which was the important factor.

From some work done in the chemical and bacteriological laboratories at Iowa State College, it was thought that the undissociated molecule of the alkali was an important factor. If this were true, the addition of a neutral salt (sodium chloride) to the alkali test solution would give a lowering of the disinfection time of the test organism with practically no change in alkalinity as measured by the pH. The results were as anticipated (5, 6). With this information at hand, it was thought that additional data might be secured concerning the type of action involved in these tests

if other neutral sodium halides were used, since all would present a common ion effect. It seemed logical that these salts would act as accelerants by decreasing the ionization of the alkali and thus decrease the solubility of the undissociated alkali molecule. If the undissociated molecule were more toxic than its ions, either directly or because of its greater penetrative power, then the increased toxicity should result in decreasing the killing times. If this assumption were true, it was hoped that some quantitative evidence might be secured which would show a definite relationship between the increased concentration of the undissociated alkali and the reduction in killing times.

While it was realized that the mass-action law is subject to considerable criticism, it was hoped that it might serve as a simple tool to express numerically the change in concentration of the undissociated molecule of sodium hydroxide when different concentrations of the same neutral salt, as well as when the same concentration of different neutral salts were added to a given concentration of alkali. Sodium salts were used in each instance in order to have the same common-ion ( $\text{Na}^+$ ). Table 5 represents a compilation of these data. These calculations were made substituting the proper

values in the following formula,  $\frac{C_{\text{Na}^+} \times C_{\text{OH}^-}}{C_{\text{NaOH}}} = K$ . This was a relatively

simple matter since the concentration in mols of both alkali and neutral salts were known. The percentage of ionization of the salts in the concentrations added, could not be secured directly in every instance from the tables in various handbooks available. To get this information, curves were constructed by plotting mols against percentage of ionization from the data in the handbooks. The percentage of ionization was then read from these curves for the different concentrations of the salts used.

Data concerning the effectiveness of sodium carbonate and tri-sodium phosphate when added to sodium hydroxide, respectively, were available from work done in these laboratories, but as no dependable dissociation tables were available, calculations were omitted (5).

In table 5, column 6, the killing times are not those of individual tests, but an average of two or more tests. The data in series 2 and 4 were taken from some previous work done in these laboratories (5, 6). The other data, excepting the constants in columns 7 and 8, are self-explanatory.

The constants, ( $K_1$ ) and ( $K_2$ ) in columns 7 and 8 were calculated by multiplying the concentrations of the undissociated alkali and the killing times (columns 4 and 6) and the concentration of the hydroxyl-ions and the killing times (columns 5 and 6). Examination of the constant ( $K_2$ ) shows that there is no close general agreement between the concentration of the hydroxyl-ions and the killing times secured. However (considering  $K_1$ ) the relationship between the concentration of the undissociated sodium hydroxide and the respective killing times is very close, excepting in the instance of the sodium iodide-hydroxide mixture (series 6), which will be discussed a little later. Attention is called to series 2, 3, and 4, where varying concentrations of sodium chloride were added to the same concentration of sodium hydroxide. These constants are in very close agreement with that secured when 0.25M sodium hydroxide alone was used. The con-

TABLE 5. *The relation of killing time to the calculated concentration of OH ions and the undissociated NaOH*

1	2	3	4	5	6	7	8
Series	Test solutions	Percent- age ion- ization of salts added (18°C.)	Concen- tration of undisso- ciated NaOH in mols	Concen- tration of OH ions in mols	Killing time (minutes)	K <sub>1</sub> Cols. 6 x 4	K <sub>2</sub> Cols. 6 x 5
1	0.25M NaOH	88.5	0.0288	0.2213	42.5	1.225	9.405
2	0.25M NaOH + 0.17M (1%) NaCl	82.8	0.04	0.210	30.6	1.224	6.426
3	0.25M NaOH + 0.342M (2%) NaCl	79.3	0.0525	0.1975	23.25	1.221	4.592
4	0.25M NaOH + 0.51M (3%) NaCl	77.4	0.064	0.1860	19.9	1.274	3.701
5	0.25M NaOH + 0.342M NaBr	79.2	0.0525	0.1975	23.25	1.221	4.592
6	0.25M NaOH + 0.342M NaI	79.8	0.0535	0.1965	18.0	0.963	3.537
7*	0.25M NaOH + 0.342M NaF	69.1	0.059	0.221	21.0	1.239	4.641

\*The calculations in this series (fluoride-hydroxide mixture) need some explanation. It was shown by titration of the mixture against a standard acid that the alkalinity was increased (0.04N) making the fluoride-hydroxide mixture 0.29M instead of 0.25M, that of the original alkali. The pH of this mixture was slightly higher as shown in table 4. This increased alkalinity was caused by the hydrolysis of the sodium fluoride. On this basis, the concentration of the undissociated alkali was calculated (column 4).



stants for the bromide-hydroxide mixture (series 5) and the fluoride-hydroxide mixture, when the correction is made for the increased alkalinity caused by hydrolysis (series 7), are in very close agreement with those of the first four series. The case of the iodide-hydroxide is the only marked deviation. The conclusion is that the concentration of the undissociated molecule of alkali is a factor of major importance in this type of disinfection.

The case of the sodium iodide-hydroxide mixture (series 6) is somewhat difficult to explain. The killing time of this mixture is far lower than the limits of experimental error would permit when compared to similar mixtures of sodium chloride, bromide, and fluoride. It should be recalled that in the discussion of technique, it was mentioned that when the five cc. portions were withdrawn at different time intervals from the disinfection flask, neutralized in dilute sulfuric acid (methyl orange), the killing time was lowered to eight minutes. Free iodine was detected (starch test). This difficulty was overcome when the portions withdrawn were made just acid to phenolphthalein. No free iodine was detected (starch test) even after a period of several hours. However, the killing time was much lower and cannot be explained by the slightly greater ionization of the sodium iodide.

Several explanations are suggested for this increased germicidal efficiency. It may be considered that the sodium iodide in acting as an accelerant may form a more toxic compound (sodium iodate) with the sodium hydroxide. Some recent data (unpublished) points that sodium iodate is very toxic in an alkali test solution. Sodium iodate may be prepared by adding finely divided iodine to hot concentrated solution of sodium hydroxide.

In this case, it is possible that when the first part of the five cc. portions was added to the dilute sulfuric acid for neutralization, hydriodic acid was formed. The amounts formed decreased as the amounts of the test solution increased and the mixture approached neutralization. Hydriodic acid is very easily oxidized even by oxygen of the air, and, undoubtedly, the free iodine produced in the first experiments with this mixture was formed in this manner. The free iodine remained in the somewhat acid medium (just alkaline to methyl orange), while in the more alkaline medium (just acid to phenolphthalein) none was detected by the starch test. Since the killing time in the latter experiments was still much less than that of the chloride and bromide mixtures, it is possible that the freed iodine was converted into minute amounts of sodium iodate, which was the effective agency in lowering the killing time. Not much consideration is given this suggestion as it is hardly plausible that any sodium iodate was formed under the conditions of the experiment.

A second explanation may be advanced that the sodium iodide increased the "activity" of the sodium hydroxide by producing a larger amount of undissociated alkali than anticipated by the method of calculation employed, and hence, accounts for the greater reduction in killing time. However, no data are available bearing directly on this suggestion.

Still another suggested explanation is that some of the free iodine, liberated at the zone of neutralization by oxidation of hydriodic acid produced when the first part of the five cc. portions of test solutions were being neutralized in the dilute sulfuric acid or possibly the hydriodic acid may have been absorbed and combined with the injured walls of the bacterial



cells. This would undoubtedly be quite germicidal. In this manner, it is possible that the amount of iodine free in the solution was lowered to such an extent that none was detected by the starch test, which is considered quite delicate. It seems reasonable to assume that the adsorption properties of the cell walls would be greatly increased after the alkali treatment and, consequently, more susceptible to the toxic action of the iodine or hydriodic acid. It is thought that this theory is worthy of more consideration.

#### SUMMARY AND CONCLUSIONS

1. Sodium halides (NaI and NaF) at the concentrations and temperatures employed had decided germicidal effect in 50 minutes on the bacterial organisms used in the disinfection tests. Sodium fluoride was most effective.

2. Sodium halides added to sodium hydroxide increased greatly the germicidal efficiency of the sodium hydroxide test solution. Addition of sodium iodide produced an abnormally high toxic effect.

3. A preliminary soaking of the test organisms in the different sodium halide solutions did not affect the resistance of the bacteria to sodium hydroxide disinfection.

4. The pH values for all alkali test solutions employed when measured by potentiometric methods were in the same range (12.83 to 12.92). The fluoride-hydroxide mixture appeared to be slightly more alkaline.

5. The assumption that the undissociated molecule of alkali is a very important factor in alkali disinfection appears to be justified. By application of the mass-action law and the common-ion effect, remarkable agreement was obtained between the anticipated calculated reductions of killing times and those obtained from experimental data. The iodide-hydroxide mixture appears to be the only exception.

6. Of the several theories suggested for the abnormal toxicity of the iodide-hydroxide mixture, the adsorption of free iodine at the zone of neutralization or of hydriodic acid by the bacterial cells is held to be the most tenable.

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# THE ACTION OF A TRANSVERSE ELECTROSTATIC FIELD UPON A PROPANE FLAME

J. K. McNEELY<sup>1</sup>, O. A. BROWN<sup>2</sup> AND C. D. CROSNO

*From the Electrical Engineering Laboratory, Iowa State College*

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Since 1928, the action of flames in transverse electrostatic fields has been intensely studied in this laboratory. This paper describes the methods and apparatus used and presents the results obtained through the use of a propane flame. Another paper will report similar studies using other flames. It is hoped that the results obtained will contribute to the theory of ionization and atomic structure.

## BEHAVIOR OF THE FLAME WITH INCREASING ELECTROSTATIC FIELD INTENSITY

The height of the flame, the distance apart of the electrodes, the humidity, and the value of the applied voltage are all factors influencing flame behavior. However, in each experiment as the voltage is raised, a definite sequence of action always follows. In experiments in which the flame is shortened by the establishing of a voltage between the electrodes, the flame slowly regains its former height as the plates lose their charge after the voltage is removed.

A high voltage alternating current field produces a combination of the effects produced by reversing the polarity of the plates using a high voltage unidirectional current.

The effects produced on the flame by the negative and positive plates were not altered by the grounding of either plate. The effects at the negative and positive plates, however, were quite different.

In 1801, Volta showed that the leaves of a gold-leaf electroscope diverge when burning charcoal is brought in contact with the knob. The incandescent particles in the flame are evidently carbon and, bearing charges, are attracted to the positive or to the negative plate. The application and gradual increase of the voltage will cause the flame to shorten and become broader without at first any noticeable bending.

## APPARATUS AND PROCEDURE

Unidirectional voltage was employed for these experiments. Each end of the high tension winding of a transformer was grounded through a Kenotron tube; the tubes were so connected that ground potential became the positive terminal, and the center tap of the transformer became the negative terminal. Approximately 50,000 volts d-c. R. M. S. value could be impressed upon the electrodes with 220 volts a-c. across the primary of the transformer. Liquefied propane gas ( $C_3H_8$ ) under reduced pressure

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<sup>1</sup>J. K. McNeely, Professor of Electrical Engineering, Iowa State College.

<sup>2</sup>O. A. Brown, Assistant Professor of Electrical Engineering, Iowa State College.

## PLATE I

Fig. 1. Propane flame at negative plate. Voltage 10,400. Flame is attracted to negative plate, while a bright bulge appears on side toward plate.

Fig. 5. Propane flame at positive plate. Voltage 18,100. Flame is deflected toward negative plate.



PLATE I

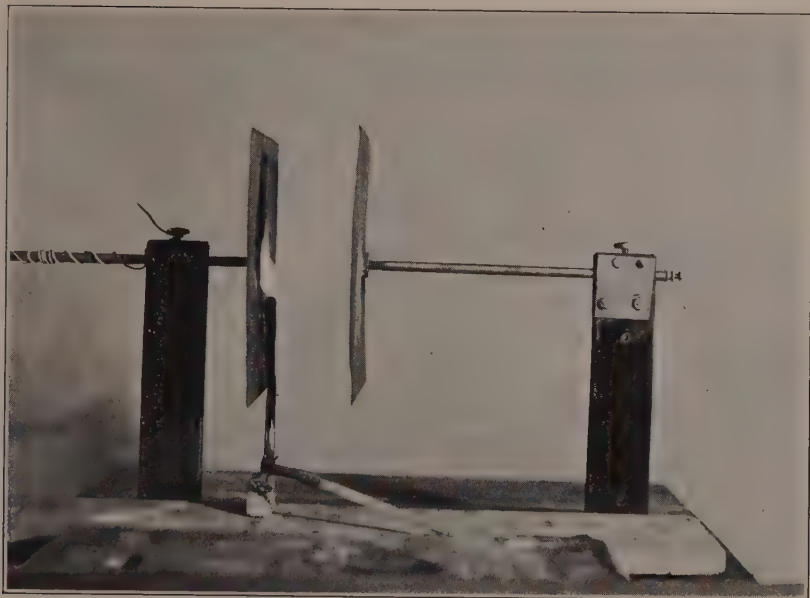


Fig. 1

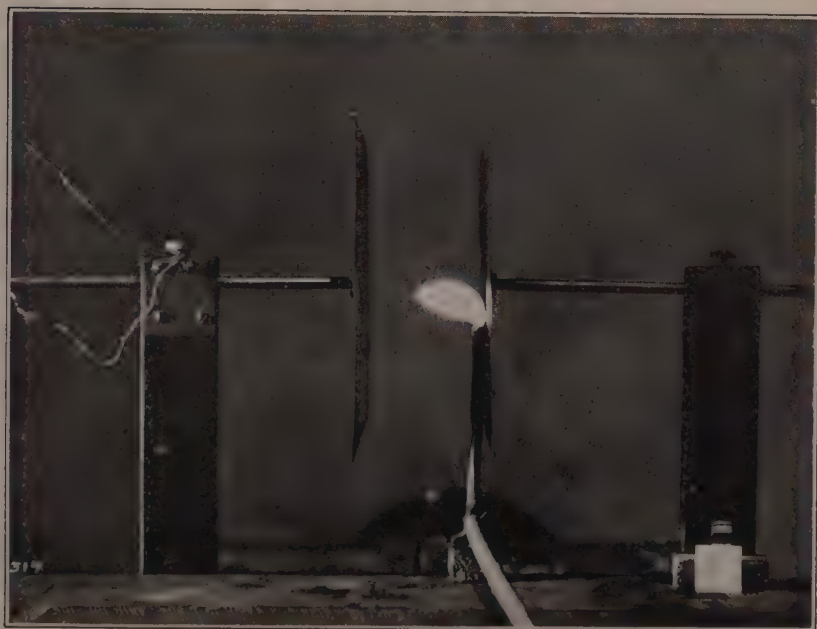


Fig. 5



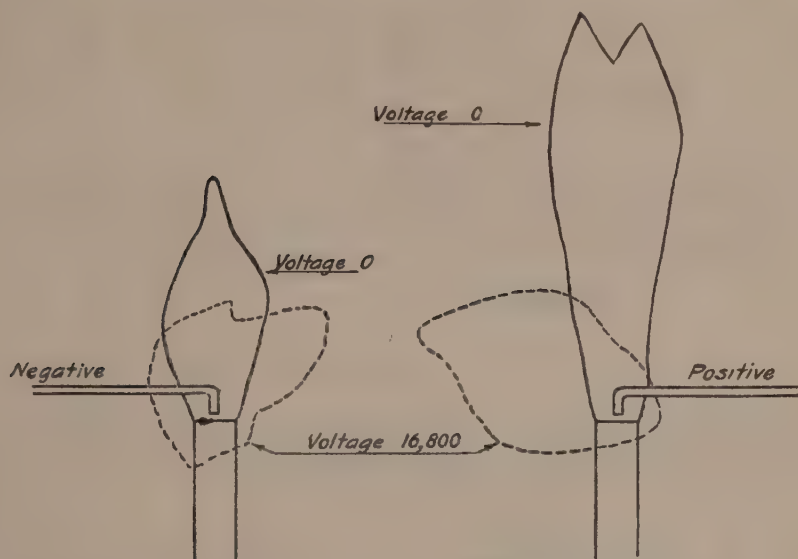


Fig. 2. Two propane flames, one positive and one negative are drawn toward one another. Increasing the voltage will extinguish the positive flame.

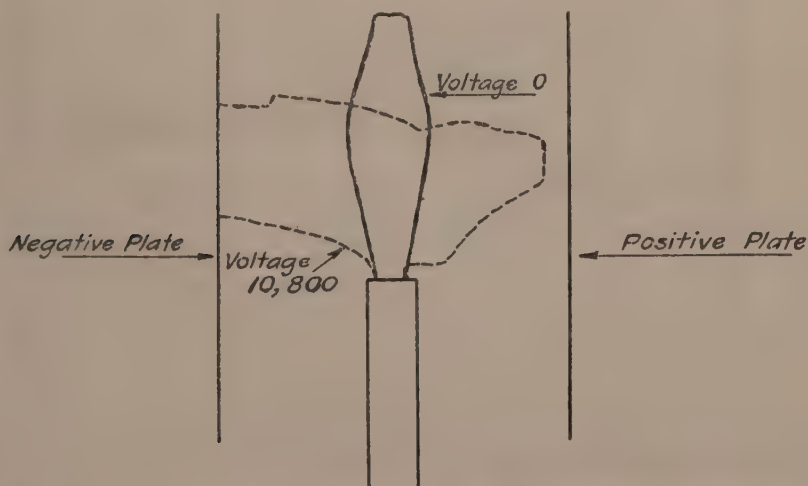


Fig. 3. Propane flame midway between plates. Voltage of 10,800 extends flame to negative electrode and close to, but not touching positive electrode.

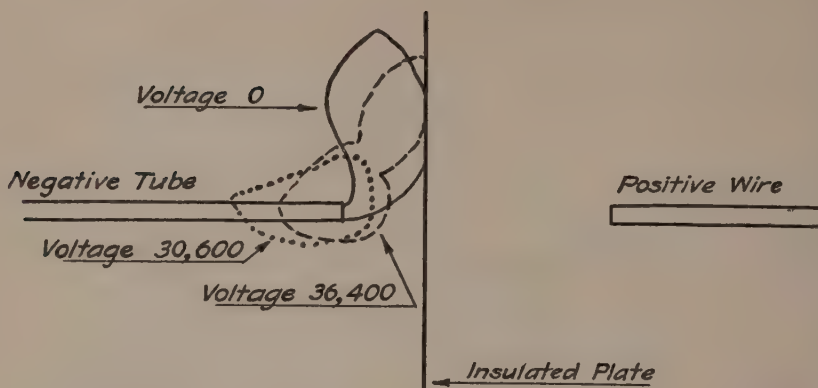


Fig. 4. Propane flame burning from a negative tube against an insulated plate. At a voltage of 30,600 the flame burns back over tube. With a voltage of 36,400, the flame again burns against insulated plate.

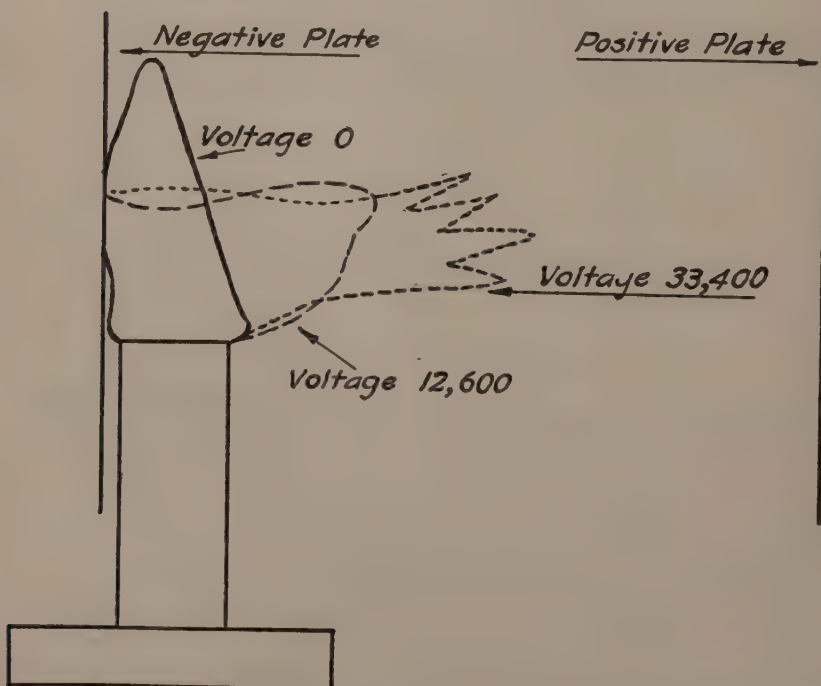


Fig. 6. Gasoline flame against negative plate. Increasing voltage causes increased deflection of flame toward positive plate.



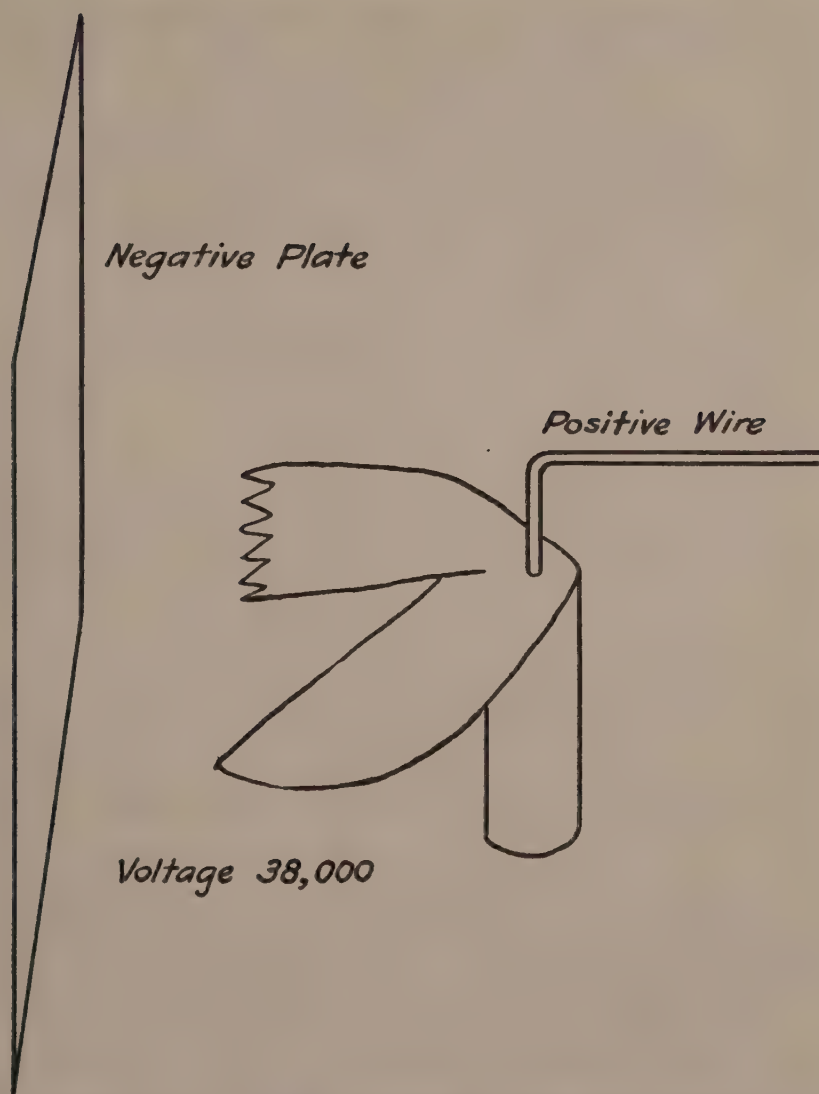


Fig. 7. Gasoline flame. A voltage of 38,000 causes positive flame to divide into two portions which apparently repel one another.

was fed to the burner. Propane was also bubbled through a solution of borax in wood alcohol and sulphuric acid for the purpose of giving visible negative ions in the flame. The burner of the propane gas was either a lava tip in the end of a copper tube or a small glass tube. The electrodes were a pair of copper plates twenty-two centimeters square placed parallel to each other at variable distances. These plates were placed vertically in a specially constructed bakelite holder. It was discovered that the application of voltage tended to shorten the flame; the flame height was measured before each experiment by keeping the valve setting and pressure fixed. The burner was insulated from ground by a rubber tube through which the gas passed.

The burner was placed between the electrodes, which were from eight to twenty centimeters apart, and the flame was adjusted to the desired height. Then the voltage between the electrodes was gradually raised by increasing the field current of the sine wave generator supplying the voltage across the primary of the transformer.

#### FLAME NEAR NEGATIVE PLATE

At 4,600 volts the flame is approximately one-half its original height. With this comparatively low voltage the heated particles of the flame do not attain horizontal speed sufficient to bend the flame, but they do cause a considerable broadening of it. As the voltage is increased above 4,600, a bulge appears in the side of the flame toward the positive plate. This bulge becomes wider with the rise in voltage until at 10,000 volts it attains its maximum width. The portion of the flame extending toward the positive plate is bright, but the tip of the flame which burns against the negative plate is blue and almost colorless. At a critical voltage, depending upon the original height of the flame, the white bulge drops rapidly back, and the whole flame burns in ripples against the negative plate with the same almost colorless flame. The height of the flame is now slightly increased while its cross section is decreased. Arcs form between the tops of the plates at 28,000 volts, preventing a further increase in voltage.

#### FLAME MIDWAY BETWEEN PLATES

The burner was placed between the electrodes, which were from eight to twenty centimeters apart, and the flame was adjusted to some desired height. Then the voltage between the electrodes was gradually raised by increasing the field current of the sine wave generator supplying the voltage across the primary of the transformer.

As the voltage is raised to a certain point, the flame flattens out more and more into a plane perpendicular to the plates. It is fan shaped; the side extending toward the negative plate being much the larger. When a critical voltage is reached, the flame ceases to be steady and vibrates in its former path from side to side.

The rate of vibration is not constant, but it seems to increase with the rise in voltage. The flame appears to be oscillating about a flat elliptical path whose major axis is along an imaginary line from the top of the burner to the center of the top of the negative plate. During this experiment the flame retains its original brightness. With a voltage of 13,800, an arc forms through the flame and rises to the top of the plates. While the arc continues, the flame rises to its original position where it burns until

the arc is extinguished; then it falls immediately to vibrating until another arc forms.

#### FLAME NEAR THE POSITIVE PLATE

The flame is placed near the positive plate. The flame shortens rapidly from its height of twelve centimeters as the voltage is applied until at 3,450 volts it is only about seven centimeters high. Raising the voltage will bend the flame toward the negative plate. It is not a smooth flame, but turbulent as if it were blown by a strong wind. The tip is rough and composed of many points. The agitation increases with the rise in voltage until intermittent arcs form through the flame at 21,600 volts.

#### ACTION OF A GASOLINE FLAME IN THE FIELD

A test tube containing waste soaked in gasoline was used as a burner. With the plate electrodes sixteen centimeters apart and a flame eight centimeters high, the burner was placed against the center of the negative plate. When the voltage was applied, the flame divided into two parts. The larger part extended toward the grounded plate and the other was attracted to the negative plate. With an increase in voltage, it retained approximately the same proportions, although the edges of the flame became sharper. The brightness of the flame is not affected by the field, even with a voltage of 40,000.

#### RESULTS USING A FLAME CONTAINING BORIC ACID

The flame was given a green tinge by passing the propane gas through a solution of borax dissolved in wood alcohol and sulphuric acid. With the exception of the changes of color, the physical action of the flame was identical with that for a similar experiment using propane not containing boric acid.

The flame was placed at the center of the negative plate. With no voltage, the flame was yellow with a green tinge at the outside. When a low voltage was applied, the flame broadened and turned a more decided yellow. The tip which extended toward the positive plate became extremely bright. At some critical voltage, the top of the flame ceased to be yellow and extend out from the plate. It became green and flattened closely against the plate.

The flame was moved to the edge of the negative plate. Until the voltage was raised to the point at which the flame began to burn against the plate, it was all yellow; above this voltage the flame was green except for a yellow tip which extended toward the positive plate.

#### SUMMARY

The gasoline and propane flames have characteristic actions at the electrodes, the action depending upon the polarity of the electrode. The flames are repelled as a whole from the positive plate.

There is a characteristic and simultaneous change of color and action of the flame in an electrostatic field as the voltage rises.

These flame actions may be explained by the following theories:

(a). That ions produced in the flame are of two types: one is positive and incapable of assuming a negative charge; the other is free to take either charge.

(b). The influence of the field on the flame is caused by the ions in the flame. Ionization is an essential part of the process of combustion of propane and gasoline and probably of all hydro-carbons.

(c). Any difference of flame action at either a flat or edged electrode is because of the greatly increased acceleration of ions from the edged electrode.

It is a well known fact that certain atoms or molecules are incapable of forming ions having either positive or negative charge, but can form only one type; others have the property of becoming ions of either charge. The results of these experiments may be explained in part by the assumption, that part of the incandescent ions formed in the flame are positive ions incapable of becoming negative, and the remainder of the incandescent particles are free to take either charge. Hence, in the experiment shown by figure 3 with the flame midway between the plates, those ions with a positive charge and incapable of being negatively charged are immediately drawn to the negative plate. Those not confined to the one charge may be positive or negative, either having this charge when they were formed, or having acquired it immediately thereafter. Those of negative charge approach the positive plate until they lose their charge in the vicinity of the plate, because of the excess of positive ions there, that is, the positive space charge; then having become positive they are attracted toward the negative electrode only to become negatively charged there. Those having an original positive charge are drawn back and forth also, starting first toward the negative plate. This dual nature of the ions formed in the flame explains why the flame in contact with the negative electrode in experiments shown in figure 2 is divided and attracted toward both electrodes, whereas the flame, when burning close to the ground plate as in experiments shown in figure 1 is always repelled as a whole, away from that plate.

It has long been known that a flame was an excellent source of ionization. Until recently, however, there has been no experimental evidence that the ionization is an essential part of the combustion in certain types of flames. Bone and Townend as a result of their elaborate and thorough investigation of combustion of the hydrocarbons make the following statement: "Meanwhile, many considerations make us think that the true explanation may ultimately be found in the supposition of some degree of ionization of one or both of the reacting gases being a necessary precedent to their combustion" (1). In this connection we may well note the experiments of F. R. Weston upon the energy required to ignite a mixture of carbonic oxide (CO) and oxygen in their combining proportions with varying degrees of wetness of the mixture, since he found that the progressive drying of the mixture increased the required energy of the igniting "ionizing" spark (2). Also, he found the completeness of the combustion of a dry mixture of these gases in their combining proportions depends upon the energy of the igniting spark, that, as he says, "Such results suggest that, for a given degree of dryness of the mixture, the greater the ionizing impulse imparted to it by the igniting spark, the more complete is the resulting combustion as the flame spreads through the explosive mixture; but this point is reserved for further inspection" (3). "In this connection also it may be recalled that, when in 1894 Smithells and Dent examined their 'separator,' the flame of  $P_2O_5$  dried mixture of cyanogen with sufficient air to give a separation of the two cones, it was found that, with the cones widely separated, the upper one, in which the combustion was almost wholly that of carbon monoxide to dioxide, was immediately extinguished



by bringing a bottle of dry air over it, but on bringing the two cones closer together, a point was soon reached where it was impossible any longer to extinguish the outer cone by such means. This very interesting result, together with that previously recorded by Dixon, certainly suggested that carbonic oxide will combine directly with oxygen, provided that it is 'freshly formed' in a flame, presumably in an 'actuated' and 'ionized' condition" (4).

"G. I. Finch and J. C. Stimson, working at the Imperial College, London, have recently repeated and extended Hartley's experiments with results of considerable significance. After generally confirming Hartley's observations, they extended them over a wide range of temperature up to 850° C. The results support the idea that both hydrogen and oxygen are always 'ionized' in contact with a gold surface at temperatures above 250°, which happens to be that at which it becomes really active in promoting the catalytic combustion; and the same applies to a silver surface. It would then appear that an 'ionization' both of the combustible gas and of the oxygen proceeds concurrently with their catalytic combustion over these surfaces" (5).

"Taking the evidence as a whole, it may be said that so far as can be seen at present, the phenomena of catalytic combustion is probably dependent upon an adsorption or occlusion of the reacting gases (or at least one of them) by the surface whereby they become activated by association with it, and that in some cases such 'activation' may be considered as 'ionization' of both the oxygen and the combustible gas. The subject requires further experimental exploration, however, before its foundations will be strong enough to bear a comprehensive theory" (6).

Thus we see that the theory of ionization as a necessary process in combustion is not entirely strange or new.

The incandescent ions of the flame as used are apparently all positive or changeable; at least there appears to be no visible ions which retain their negative charge adjacent to the positive plate. The introduction of such ions into the flame might assist in the explanation of flame action. The propane gas was passed through a solution of borax in sulphuric acid and wood alcohol. The boric acid is known to give a visible green negative ion in the flame.

In test, the green ions did not appear to be negative, but they acted in all respects like positive ions—always being attracted to the negative plate. Although this scheme did not produce the desired results, it did call attention to facts which may be of value.

If the flame containing boric acid is burned from a Bunsen burner, and the air supply is gradually increased, the flame at first was mainly yellow with an outside sheath of green, but gradually loses its yellow cone and finally becomes wholly green. Thus it is seen that the flame is wholly green when combustion is complete; the yellow color might be caused by sodium from the borax, but since the color disappears with an increase in air supply, this is apparently not the cause.

When the yellow color is removed from the flame wholly or in part, leaving it completely or partly green, it is evident that the combustion has become complete, or more nearly complete, since the yellow color is the result of incomplete combustion. Using the flame containing boric acid in three experiments similar to experiments, figures 1, 2, 3, the yellow color was removed either gradually or suddenly as the voltage was increased in-

dicating that a change in electrostatic field produces a change in completeness of combustion. In experiments with the flame between the plates or at the negative plate, this change proceeded somewhat uniformly with the rise of voltage; while with the flame at the positive plate, this change occurred suddenly at some definite critical voltage depending upon the height of the flame and the distance between electrodes.

The fact that a tip of flame at lower voltages is repelled from the negative plate while the remainder, darker in color, burns against the plate has been explained upon the assumption that the ions in this tip are capable of assuming a negative charge while those burning against the electrode refuse to take on a negative charge even after they are in contact with the negative plate. Whether these positive ions retain their charge while touching the negative plate, or whether they become neutral is not shown by their action, for in either case they would rise as they do beside the electrode. Not only are the ionizing properties of the two parts of the flame different, but the degree of combustion is also different, for that portion of the flame against the plate contains no yellow, indicating complete combustion, while the tip is quite a bright yellow. An increase in voltage will destroy the tip and the yellow color simultaneously. The portion of the flame in which combustion is not complete and the visible ions capable of being negatively charged are inseparable in an electrostatic field. The part of the flame which burns against the negative electrode is in every case a flame in which combustion is complete; that portion of the flame repelled from the negative electrode in every case is a flame in which combustion is not complete. An electrostatic field capable of causing complete combustion eliminates visible ions which can be charged negatively.

Ions are more readily expelled from edges and points than from flat surfaces. Any difference of action of the flame placed against the edge of one plate as compared to one placed at the center of the plate—other conditions being equal—may be caused in part by the difference of the amounts of ions projected into it. Thus in experiments when a small flame surrounded by a copper gauze was used, the flame was not affected even with a voltage of 38,000 when it was placed midway between the plates or against the flat surface of either. With the same voltage the flame, placed against the edge of either plate was bent away from that plate. In none of the cases could an electrostatic field have any effect because the copper gauze would shield the flame from the electrostatic lines of force. Ions, however, could pass from the edge of the plate through the gauze and deflect the flame by bombarding it.

Bone, Townend, and others have recorded information of the influence of water vapor upon completeness, rapidity, and other characteristics of combustion of hydro-carbons. A certain action of the flame did not appear in several trials; in fact, it appeared only on occasions when rain was falling or when the gas had been passed through a chemical solution. Apparently water vapor has an influence upon ionization as well as combustion—probably identically the same influence in both cases.

The Cottrell method of electrical precipitation consists of passing gases containing suspended particles through an electrostatic field of the point to plate type. Ions expelled from the point source charge the suspended particles with the charge of the point source, causing them to be deposited on the plate electrode. In practice the point source is the negative, and the plate is the positive electrode. The ionic discharge is usually in the form of corona discharge.

"Smoke, or other suspended particles contained in gaseous streams, passes with considerable freedom from being precipitated through positive corona, the alternating corona, or the localized discharge" (7). "The use of negative corona is the most effective in precipitating particles in gaseous streams: and this is no doubt due to the high electronic and ionic velocities associated with this type of corona discharge or electric discharge in general" (8).

Some carbon was deposited from the flame in these experiments. A very large percentage of this carbon formed on the negative electrode. Minute particles would often pass to the grounded plate from the negative; others would fly rapidly back and forth between the plates and drift out of the range of the field. The fact that the Cottrell process deposits such previously neutral particles upon the positive plate and that the carbon from the flame invariably deposits on the negative electrode are not, as might seem at first inspection, contradictory.

The action of depositing activated carbon from a flame in which it undoubtedly exists in an ionized state, and the action of depositing suspended neutral relatively cool particles from a gas by giving them a charge from a point source, are entirely different processes.

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# A STUDY OF CROSSES BETWEEN TREBI AND THREE SMOOTH-AWNED VARIETIES OF BARLEY<sup>1</sup>

PEDRO A. DAVIS

*From the Department of Farm Crops and Soils, Iowa State College*

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Since the rediscovery of Mendel's law in 1900, there have been many critical studies of the heritable characters in *Hordeum*. From a careful perusal of the literature, however, one finds that while previous investigators agree on the manner of inheritance of many simple Mendelian characters, little work has been done on the inheritance of the quantitative characters such as yield, height of plant, number of culms, and length of spike. More information concerning the inheritance of such characters in barley is indispensable to an intelligent attack of the problems of barley improvement.

According to Hughes and Burnett (14) there is a demand for high yielding, smooth-awned varieties of barley suited to Iowa conditions. It was in view of this demand that the hybridization work with barley was projected. It was also desired to determine the nature of the inheritance of some quantitative characters in barley under the existing conditions in Iowa, as well as the relation of yield to other quantitative characters.

## REVIEW OF LITERATURE

Because of its commercial importance and its many distinct heritable characters, the barley plant has been the subject of a large number of genetic studies. No attempt in the present work is made to present a complete resume of all the published genetic investigations in barley. Only the papers which have a direct bearing on the inheritance of the characters studied are reviewed.

## ROUGHNESS OF AWNS

In 1927 Tschermak (22) gave a review of studies on barley hybridization. These studies were conducted in Germany, Sweden and Austria, in attempts to obtain increased hardiness and yield, resistance to disease, earliness and improved quality. He described, also, his own attempt to produce smooth-awned varieties.

Vavilov (24) obtained some smooth-awned plants in the  $F_2$  generation from a cross between two rough-awned parents. He assumed that

<sup>1</sup>The major part of a thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup>Instructor in Agronomy, University of the Philippines, Manila, Philippine Islands; on leave of absence to do graduate work at Iowa State College. The author gratefully acknowledges his indebtedness to Dr. J. E. Wentz for assistance and advice during the progress of the investigation, to Dr. E. W. Lindstrom for advice on genetic phases of the problem, and to Prof. G. W. Snedecor for assistance in the statistical analysis of the data.

the smooth-awned barleys were probably the product of hybridization. He found different degrees of roughness and smoothness, with no varieties entirely free of barbs throughout the entire length of their awns. In general, the smooth-awned plants bred true for smoothness. In some crosses the rough and smooth-awned plants differed in several genetic factors for awn condition. His results indicated that these genes modified the character, form and frequency of the barbs produced on the rough-awned plants.

Colin and Trouard-Riolle (4) crossed smooth-awned black barley with rough-awned Albert barley and obtained different types of  $F_1$  plants. Some plants showed both smooth and rough awns, others showed awns which were smooth for half their length and rough for the remaining half, and still others showed smooth awns only. Their results in the later generations were too complicated to permit genetic explanations.

Harlan (8) was the first American investigator to make crosses between rough and smooth-awned barley varieties. He reported that roughness of the awns was determined by a single factor, roughness being dominant. He also pointed out that all smooth-awned barleys were not equally smooth.

Hayes *et al* (13) made crosses between the varieties Lion (smooth) and Manchuria (rough) and found that the  $F_1$  plants had rough awns and the  $F_2$  generation segregated in a ratio of three rough to one smooth. All the smooth-awned  $F_2$  plants bred true in the  $F_3$  generation, but with various degrees of smoothness. Also some of the  $F_2$  rough-awned plants bred true in the  $F_3$  generation while others segregated into rough and smooth-awned plants representing all forms found in the  $F_2$  generation. Their results indicated a one-factor difference in the presence of other modifying genes brought in by the smooth-awned parent.

Griffie (7) crossed the varieties Svanhals and Lion and found that the parent varieties differed in two factors for the development of barbs on the awns. He obtained in the  $F_2$  generation twelve rough to three intermediate-smooth to one smooth-awned plant. Gene *R* alone or with *S* gave rough awns. Gene *S* in the absence of *R* produced intermediate-smooth awns. In the absence of *R* or *S* the awns were smooth like those of the smooth-awned parent. His results suggested a modifying factor which influenced the degree of smoothness of the awns to a slight degree.

Sigfusson (20) reported that roughness of barley awns is caused by two complementary factors, which he designated as *R* and *S*. The *R* factor in the absence of *S* produced the intermediate-rough awns and the *S* factor in the absence of *R* produced intermediate-smooth awns. Both factors were necessary to produce fully rough awns. The smooth awns were the result of the double recessive, *rrss*. With this assumption he obtained four phenotypes in the  $F_2$  generation in the ratio of nine rough to three intermediate-rough to three intermediate-smooth to one smooth. Sigfusson's progeny test in the  $F_3$  generation failed to show the expected segregation in some of his rough  $F_2$  plants. He attributed this discrepancy to the small number of plants tested.

#### TIME OF FLOWERING

Different indices have been used by different investigators in studies of the inheritance of earliness in cereal crops. The index of earliness used in the present study was the date of emergence of the tips of the first awns

from the boot of the plant. Harlan (9) in his studies on barley found that the emergence of the awns was a more accurate index of the date of flowering than the date of heading. He stated that the "precocity of the strain at the time of the emergence of the awn is a heritable character."

Harlan and Martini (10) in their studies on the earliness in  $F_1$  barley hybrids found that the hybrids headed quite uniformly as compared with their respective parents. The average awn-emergence date of the hybrids tended to be intermediate between the two parents. The grand average awn-emergence date of all the hybrids was more than two days earlier than the average of all the parents. The earliness of many hybrid combinations of late varieties indicated that they contained many factors for earliness. According to these investigators, earliness is closely related to the length-of-day factor.

Neatby (18) found that the time of maturity in a barley cross was probably dependent on two or more independently inherited factors.

Griffiee (7) crossed early with late heading strains and obtained early  $F_1$  plants which gave three early to one late plant in the  $F_2$  generation.

#### HEIGHT OF THE PLANT

Vestergaard (25) crossed a dwarf-like variant with a Binder barley and obtained in the  $F_2$  generation 14 individuals of the former type and 81 of the latter.

Ubisch (23) reported that the height of the culms was governed by one or more of three possible factors.

Miyake and Imai (16) obtained tall, slender  $F_1$  plants by crossing tall, slender with short, stout types. They found a ratio of three tall to one short plant in the  $F_2$  generation, indicating one main factor difference.

Miyazawa (17) described a dwarf type as a result of backcrossing the variety Golden Melon on an  $F_1$  of the cross Golden Melon  $\times$  Sekitori. He found that the homozygous dwarfs were small and sterile and that the dwarf type tended to dominate the normal type. In the  $F_2$  generation a segregation of one sterile-dwarf to two dwarf to one normal plant was obtained.

According to Fruwirth (6), Tschermak obtained intermediate  $F_1$  plants from a cross between plants with tall and short culms. A normal monohybrid ratio of one tall to two intermediate to one short plant was obtained in the  $F_2$  generation, indicating one main factor difference.

Harlan and Pope (11) presented evidence that a dwarf form behaved as a simple recessive to normal barley.

In an analysis of the inheritance of quantitative characters and linkage in barley, Neatby (19) studied the height of plants in 228 lines and determined the mean value and standard deviation for each line. In his results the number of factors concerned in the inheritance of height was not clear. He suggested that using the standard deviation of the parents as an index of homozygosity, the operation of four genetic factors was indicated.

#### NUMBER OF CULMS PER PLANT

Harlan (9) found that the number of culms per plant in barley seemed to be a varietal character but was greatly affected by environment.

In regard to tillering, Barbaeki (1) reported (*vide* English summary)



that the differences between the parental forms examined by him were dependent upon two cumulative factors.

#### LENGTH OF SPIKE

A paper by Barbaeki (1) contains evidence (*vide* English summary) that the length of the spike in barley is dependent upon two cumulative factors.

The mode of inheritance of the length of internode in the spike, or density of the spike, has been investigated by several investigators. Their results are too complicated to admit satisfactory genetic interpretation.

#### YIELD

The literature on barley genetics and breeding does not, as far as the author is aware, contain data on the inheritance of plant yield. A number of investigators have reported on plant yields in other crops. The present paper reviews only a few of these reports which typify the studies on this character.

Engledow and Wadham (5) found that yield in cereals is controlled by a great number of factors which are themselves complex and not clearly understood.

In an investigation of the inheritance of yield and protein content in wheats, Clark and Quisenberry (3) found that the mean yield of the hybrids was intermediate between the two parents and that the  $F_2$  plant yields were more variable than the plant yields of either parent.

Jones (15) reported that yield in rice is the result of multiple factors and that the  $F_2$  hybrids gave higher yields and were more variable than their parents. The  $F_3$  yields were lower than those of either parent and were more variable.

#### CORRELATION OF CHARACTERS

Neatby (18) found a positive correlation of  $0.4977 \pm 0.0368$  between the height of  $F_2$  plants and the mean heights of their  $F_3$  progenies in a cross between the varieties Guy Mayle and Canadian Thorpe.

In a later study on the inheritance of quantitative characters and linkage in barley, Neatby (19) found the correlation coefficient between the date of heading of  $F_2$  plants and the mean date of heading of their  $F_3$  progenies to be  $0.5953 \pm 0.0286$ . He obtained a correlation coefficient between the mean height and mean earliness in the  $F_3$  lines of  $0.4559 \pm 0.0344$ .

Tedin (21) pointed out that if the correlation within the pure lines crossed is not known, correlation in a mixed population does not give an indication of the genetic relationships.

#### MATERIALS

The barley varieties used in the present study were Trebi, Comfort, Glabron and Velvet. Trebi was used as the male parent and the other three as female parents. These varieties were originally obtained from the Minnesota Agricultural Experiment Station and had been grown by the Farm Crops Section of the Iowa Agricultural Experiment Station.

Trebi is a six-rowed, white, high yielding, rough-awned variety. According to Harlan *et al* (12) it was produced from a single plant selection

at the University of Minnesota in their cooperative studies with the Bureau of Plant Industry, United States Department of Agriculture. The barley from which it was selected was originally imported by the United States Department of Agriculture from Samsoun, Asiatic Turkey. Although Trebi is a vigorous, high yielding barley over a large area of country it has some drawbacks. It has the rough awned character which makes it disagreeable to handle. It is also susceptible to *Helminthosporium sativum* P. K. B.

Comfort, Glabron and Velvet are all six-rowed, smooth-awned varieties. These were all produced by the Minnesota Agricultural Experiment Station. Comfort and Velvet were developed from crosses between Smooth Awn and Luth. They were found to be resistant to the *Helminthosporium* disease and fairly good yielders. Glabron was developed from a cross between Smooth Awn and Manchuria. It has been reported as the highest yielding smooth-awned variety in Minnesota, and as possessing a stiffer straw than Trebi.

#### METHODS

The  $F_1$  generation was grown in the greenhouse in the winter of 1928-29 under temperatures of 65 to 85 degrees F. Artificial light was used to lengthen the days in the greenhouse.

The  $F_2$  and  $F_3$  field cultures were grown on the Iowa State Agricultural Experiment Station grounds, where the soil is fairly uniform. The  $F_2$  and  $F_3$  generations and their parents were planted in rows spaced one foot apart. The seeds were planted three and one-half inches apart in the row. Five border rows were planted around the field. All field operations were made as uniform as possible.

In the  $F_2$  generation, considerable variation was noted in the number and size of barbs on the rough awns and an attempt was made to distinguish different classes of rough awns. After some study and several attempts to establish different grades of roughness it was decided that all awns would be classified as either rough or smooth. By smooth awns is meant awns on which the barbs are wanting, except for a few small ones at the extreme tip. They appear as smooth as the awns of the smooth female parents. In all cases the roughness of the awns was determined by the aid of a binocular microscope.

The criterion used in the study of the time of flowering was the date of emergence of the first awns. The plants were examined daily in the field at practically the same hour and labeled with the flowering date.

The height of the plants was measured from the base of the culms to the tip of the uppermost spike. The plants were pulled individually at maturity and measurements taken in centimeters.

The number of culms per plant was recorded in the field at harvest time and subsequently checked in the laboratory when the height was measured. Only culms which bore heads were recorded as the number of culms of the plant.

The length of the average spike was measured in tenths of centimeter. The measurement was taken from the base of the spike to the tip of the uppermost grain. The average length of all the heads or spikes on each individual plant was taken as the spike length for that particular plant.

After all the characters were measured, the heads were stored until threshing time. The  $F_1$  and  $F_2$  plants and their parents were hand threshed

and their weights recorded to the nearest tenth gram. In the  $F_3$  generation, the individual plants were threshed in a thresher devised by the writer.

Two of the five crosses were carried into the  $F_3$  generation. The  $F_2$  plants were selected at random in each of these two crosses for planting the  $F_3$  generation.

All data in the  $F_3$  generation were recorded on punch cards and machine methods were used in calculating all constants. Means, standard deviations and simple and multiple correlation coefficients were calculated through the use of machine factoring as described by Brandt (2), and correlation and machine calculation methods of Wallace and Snedecor (26).

## RESULTS

Excepting the correlation studies, the results are presented by characters. The data for the two or three generations of all crosses are collected together and discussed under each of the characters studied.

### ROUGHNESS OF AWNS

The roughness of awns was studied in the  $F_1$  and  $F_2$  generations in all the five crosses. Two of the crosses, No. 10 (Glabron x Trebi) and No. 28 (Velvet x Trebi) were carried through the  $F_3$  generation.

*F<sub>1</sub> and F<sub>2</sub> data.* In all five crosses the  $F_1$  hybrids had rough awns. The  $F_2$  families segregated into what appeared to be ratios of three rough-awned plants to one smooth-awned plant. The  $F_2$  data are compared with the theoretical 3:1 ratio in table 1.

TABLE 1. *Number of rough and smooth-awned plants in the  $F_2$  generation compared with the theoretical 3:1 ratio*

Cross no.	Actual number		Theoretical		Dev.	P.E.
	Rough	Smooth	Rough	Smooth		
10 (Glabron x Trebi)	221	78	224.2	74.8	3.2	0.6
23 (Comfort x Trebi)	250	117	275.3	91.7	25.3	4.5
28 (Velvet x Trebi)	360	95	341.3	113.7	18.7	3.0
32 (Glabron x Trebi)	129	38	125.3	41.7	3.7	0.9
33 (Glabron x Trebi)	205	78	212.3	70.7	7.3	1.9

These data fit reasonably well the theoretical 3:1 ratio and without  $F_3$  progeny tests one would conclude that the roughness of awns, as classified in these studies, was controlled by a single pair of factors. The  $F_3$  progeny tests, however, showed that the  $F_2$  ratios could not have been caused by the segregation of a single pair of factors. About two-thirds of the  $F_3$  progenies from smooth-awned  $F_2$  plants segregated in ratios approximating three smooth-awned plants to one rough-awned plant. This suggested the presence of an inhibitor. The  $F_2$  data were then compared with the theoretical 13:3 ratio as shown in table 2.

With the exception of one cross (No. 23) the  $F_2$  data fit the 13:3 ratio almost as well as the 3:1 ratio. Unfortunately, cross No. 23 was not tested in the  $F_3$  generation.

TABLE 2. *Number of rough and smooth-awned plants in the F<sub>2</sub> generation compared with the theoretical 13:3 ratio*

Cross no.	Actual number		Theoretical		Dev.	Dev. P.E.
	Rough	Smooth	Rough	Smooth		
10 (Glabron x Trebi)	221	78	242.9	56.1	21.9	4.8
23 (Comfort x Trebi)	250	117	298.2	68.8	48.2	9.6
28 (Velvet x Trebi)	360	95	369.7	85.3	9.7	1.7
32 (Glabron x Trebi)	129	38	135.7	31.3	6.7	1.9
33 (Glabron x Trebi)	205	78	229.9	53.1	24.9	5.6

*F<sub>3</sub> data.* Following up the suggestion of the presence of an inhibitor, it was assumed that in the two crosses tested in the F<sub>3</sub> generation there was present dominant genetic factor for smooth awns and a dominant factor which inhibited smooth awns. The genetic constitution then of the parents in these crosses might have been AAii (smooth-awned) and aaII (rough-awned). Here A is the dominant factor for smoothness and I is the dominant inhibitor. The cross might be represented in the F<sub>1</sub> and F<sub>2</sub> generations together with the breeding behavior in the F<sub>3</sub> generation as follows:

P<sub>1</sub>.....AAii (smooth) x aaII (rough)

F<sub>1</sub>.....AaIi (rough)

F<sub>2</sub> and F<sub>3</sub> as follows:

Phenotypes	Genotypes	Breeding behavior in F <sub>3</sub>
13 rough	1 AAII	Breed true
	2 AAii	3 rough : 1 smooth
	2 AaII	Breed true
	4 AaIi	13 rough : 3 smooth
	1 aaII	Breed true
	2 aaIi	Breed true
	1 aaii	Breed true
3 smooth	1 AAii	Breed true
	2 Aaii	1 rough : 3 smooth

This gives an F<sub>3</sub> progeny distribution as follows:

From rough-awned F<sub>2</sub> plants—

7 Breeding true for rough awns

4 Segregating in ratio of 13 rough : 3 smooth

2 Segregating in ratio of 3 rough : 1 smooth

From smooth-awned F<sub>2</sub> plants—

1 Breeding true for smooth awns

2 Segregating in ratio of 3 smooth : 1 rough

The distribution of F<sub>3</sub> progenies from rough-awned and from smooth-awned F<sub>2</sub> plants are shown in tables 3 and 4 as compared with the numbers



expected on the basis of a dominant factor for smooth awns with a dominant inhibitor. The  $F_3$  segregating families were classified under 13:3 or 3:1 ratios depending upon which one of these ratios the numbers approached closest as judged by the deviations divided by the probable errors. The fit of the actual numbers obtained to the theoretical numbers is surprisingly close.

TABLE 3. *Distribution of  $F_3$  progenies grown from rough-awned  $F_2$  plants*

Cross No.	Seg. 3:1		Seg. 13:3		Total seg.		Non-seg.		$X^2$	P
	Act.	Theo.	Act.	Theo.	Act.	Theo.	Act.	Theo.		
10	18	19	39	38	57	52.0	55	60.0	0.50	Very high
28	13	13	26	26	39	47.1	63	54.9	1.20	0.56
Total	31	32	65	64	96	99.1	118	114.9	.12	Very high

TABLE 4. *Distribution of  $F_3$  progenies grown from smooth-awned  $F_2$  plants*

Cross no.	Seg. 1:3		Non-seg.		Dev. P.E.
	Act.	Theo.	Act.	Theo.	
10	18	21.3	14	10.7	1.8
28	29	28.0	13	14.0	0.5
Total	47	49.3	27	24.7	

The reasonably close fit of the  $F_2$  data to the theoretical 13:3 ratio and the close fit to theory of the  $F_3$  progeny ratios strongly support the inhibitor hypothesis. A study of the ratios of rough to smooth-awned plants in the segregating  $F_3$  progenies, however, indicates that there is some other factor affecting the roughness and smoothness of the awns. Although the families are small and one could not expect close fits to theory in the individual families there are a number of families in which the ratios are too far from theory to be accounted for by chance. The data for the  $F_3$  segregating families are shown in tables 5, 6 and 7. Although the segregating families fell into about the right proportion of 13:3 and 3:1 ratios by the method of classification used, it is evident from the data in tables 5, 6 and 7 that a number of the families present odd ratios. This is particularly true in table 7.

*Application of Inhibitor Hypothesis to Data of Previous Investigators.* The results of Griffee (7) and Sigfusson (20) can be explained on the basis of the inhibitor hypothesis if it is assumed that in their crosses they had the dominant factor *A* for smooth awns in both parental plants in all their crosses and that their factors *R* and *S* were dominant inhibitors of this factor. Sigfusson's crosses, for example, could be represented as follows:

$P_1$ ..... $\Delta$ ARRSS (Rough) x  $\Delta$ Aarrss (Smooth)  
 $F_1$ ..... $\Delta$ ARrSs (Rough)

F<sub>2</sub> and F<sub>3</sub> as follows:

Phenotypes	Genotypes	Breeding behavior in F <sub>3</sub>
9 rough	1 AARRSS	Breed true
	2 AARRSs	3 rough : 1 intermediate-rough
	2 AARrSS	3 rough : 1 intermediate-smooth
	4 AARrSs	9 : 3 : 3 : 1
3 Intermediate-rough	1 AARRss	Breed true
	2 AARrss	3 intermediate-rough : 1 smooth
3 Intermediate-smooth	1 AArrSS	Breed true
	2 AArrSs	3 intermediate-smooth : 1 smooth
1 Smooth	1 AArrss	Breed true

The results of Harlan (8) and Hayes *et al* (13) can be explained by assuming the presence of the dominant *A* in all their parental plants and the segregation for one dominant inhibitor in the F<sub>2</sub> generation.

TABLE 5. Ratios of rough to smooth-awned plants in F<sub>3</sub> segregating families grown from rough-awned F<sub>2</sub> plants (F<sub>3</sub> families assumed to be segregating in a ratio of 13:3)

Family No.	Rough	Smooth	Total	Family no.	Rough	Smooth	Total	
X10— 38	39	6	45	X10—171	38	4	42	
46	40	6	46	173	35	2	37	
50	41	5	46	175	39	4	43	
55	36	9	45					
58	45	2	47	Total	Act. Theo.	1412 1347.1	246 310.9	1658
65	39	8	47					
67	38	5	43	X28—255	31	7	38	
69	39	6	45	257	42	4	46	
73	41	4	45	262	36	5	41	
79	38	5	43	264	29	3	32	
80	42	4	46	267	34	3	37	
83	31	7	38	276	38	7	45	
89	33	5	38	280	21	3	24	
90	35	9	44	284	35	6	41	
92	38	8	46	295	14	2	16	
95	31	8	39	306	39	3	42	
97	32	8	40	308	35	4	39	
99	32	4	36	313	40	5	45	
107	28	3	31	314	36	2	38	
110	36	8	44	317	37	8	45	
111	39	8	47	321	38	3	41	
118	30	3	33	323	27	5	32	
127	38	6	44	324	31	2	33	
134	41	8	49	328	35	5	40	
135	35	7	42	339	38	3	41	
139	35	9	44	361	34	7	41	
141	45	4	49	365	37	7	44	
145	29	5	34	371	35	9	44	
146	35	2	37	375	31	8	39	
155	37	4	41	380	40	7	47	
159	32	6	38	382	34	9	43	
161	37	4	41	386	33	7	40	
162	34	8	42					
163	33	7	40					
165	32	6	38	Total	Act. Theo.	880 823.9	134 190.1	1014
166	34	9	43					

TABLE 6. *Ratios of rough to smooth-awned plants in  $F_1$  segregating families grown from rough-awned  $F_2$  plants ( $F_2$  families assumed to be segregating in a ratio of 3:1)*

Family no.		Rough	Smooth	Total	Family no.		Rough	Smooth	Total
X10—	41	35	10	45	X28—	253	26	15	41
	42	28	12	40		258	33	10	43
	60	30	11	41		263	26	10	36
	102	35	10	45		266	33	9	42
	103	37	11	48		271	37	11	48
	109	31	17	48		275	28	9	37
	115	30	13	43		300	32	10	42
	121	29	9	38		305	30	9	39
	129	35	12	47		331	30	10	40
	131	32	12	44		307	29	11	40
	137	33	12	45		351	33	10	43
	142	27	9	36		363	29	15	44
	147	29	10	39		388	30	10	40
	150	24	12	36	Total	Act. Theo.	396 401.2	139 133.8	535
	151	30	12	42					
	153	31	9	40					
	170	31	10	41					
	176	35	11	46					
Total	Act. Theo.	562 573	202 191	764					

TABLE 7. *Ratios of rough to smooth-awned plants in  $F_1$  segregating families grown from smooth-awned  $F_2$  plants*

Family no.	Rough	Smooth	Total	Family no.	Rough	Smooth	Total	
X10—45	13	27	40	X28—287	9	33	42	
52	9	33	42	288	34	9	43	
54	19	29	48	293	11	29	40	
61	10	32	42	294	8	34	42	
62	8	32	40	302	18	22	40	
71	22	16	38	337	16	17	33	
77	28	16	44	340	20	19	39	
86	8	34	42	342	12	35	47	
108	11	30	41	350	28	15	43	
124	21	22	43	352	18	27	45	
132	2	42	44	357	27	13	40	
140	8	30	38	358	28	4	32	
144	12	26	38					
148	32	13	45	360	21	20	41	
156	3	40	43	362	32	12	44	
160	19	19	38	370	38	3	41	
164	33	5	38	373	21	22	43	
172	34	6	40	376	21	12	33	
				377	25	10	35	
Total	Act. Theo.	292 186	452 558	744	379	9	27	36
				381	35	5	40	
				387	2	43	45	
X28—260	2	40	42	389	8	34	42	
269	13	35	48	391	22	19	41	
278	29	13	42	393	9	34	43	
279	35	13	48					
286	8	28	36	Total	Act. Theo.	559 296.5	627 889.5	1186

Vavilov (24) obtained smooth-awned plants from the  $F_2$  generation of crosses between rough-awned parents. This would be possible by assuming that his cross was AARRss x AArrSS.

#### TIME OF FLOWERING

Table 8 shows the frequency distributions, means and coefficients of variation for the number of days from planting to flowering for each generation of the five crosses. The most interesting fact observed in this table is the earliness of the hybrids as compared with their parents. In four of the five crosses the hybrids in each generation are earlier than either of their respective parents. It is evident that in these four crosses each of the two parents contributed factors for earliness which were not present in the other. This assumption is further substantiated by the range in the  $F_2$  distributions as compared with the frequency distributions of their parents. The  $F_2$  distributions show a much greater range in maturity, and in each case the increased range is due to the earlier classes. In each of the  $F_2$  and  $F_3$  populations, plants are found ranging from the earliest plants of the earlier parent to the latest plants of the later parent. The variability of the  $F_2$  and  $F_3$  generations is further indicated by the higher coefficients of variation. The variability and wide range in the hybrid populations is especially interesting in view of the small differences in the earliness of the parents used in the crosses.

In the one cross, No. 23, the time of flowering of the  $F_1$  generation is practically the same as the earlier flowering parent. This indicates that, as in the four other crosses, earliness is dominant to late flowering. In the  $F_2$  generation, however, the range and the coefficient of variation is practically the same as those of the parents. It seems evident that the parents in the Comfort x Trebi cross did not differ as greatly in genetic factors for earliness as was the case in the other four crosses.

The results from the study of the inheritance of earliness in crosses No. 10 and No. 28 will be discussed in detail as these two crosses were carried through the  $F_3$  generation, and illustrate the behavior of quantitative characters.

*F<sub>1</sub> generation.* In cross No. 10, Glabron x Trebi, the mean difference between the two parents in the number of days to flowering was  $3.8 \pm 0.2$  days. The  $F_1$  hybrid plants flowered  $5.2 \pm 0.2$  days earlier than the male parent and  $1.3 \pm 0.1$  days earlier than the female parent. The differences between the hybrid plants and their parents are significant in the light of their probable errors.

The two parents in cross No. 28, Velvet x Trebi, showed a mean difference of  $2.6 \pm 0.3$  days in the number of days to flowering. Comparing the  $F_1$  hybrids with their parents, differences of  $2.3 \pm 0.3$  and  $5.9 \pm 0.3$  days, respectively, were obtained in favor of the hybrids. Both differences are significant.

*F<sub>2</sub> generation.* In cross No. 10, 289  $F_2$  plants were grown from six  $F_1$  plants, and in cross No. 28, 411  $F_2$  plants were grown from nine  $F_1$  plants.  $F_2$  frequency distributions for number of days from planting to flowering using a class interval of one day are given in table 9. A graphical representation of these data is presented in figure 1.

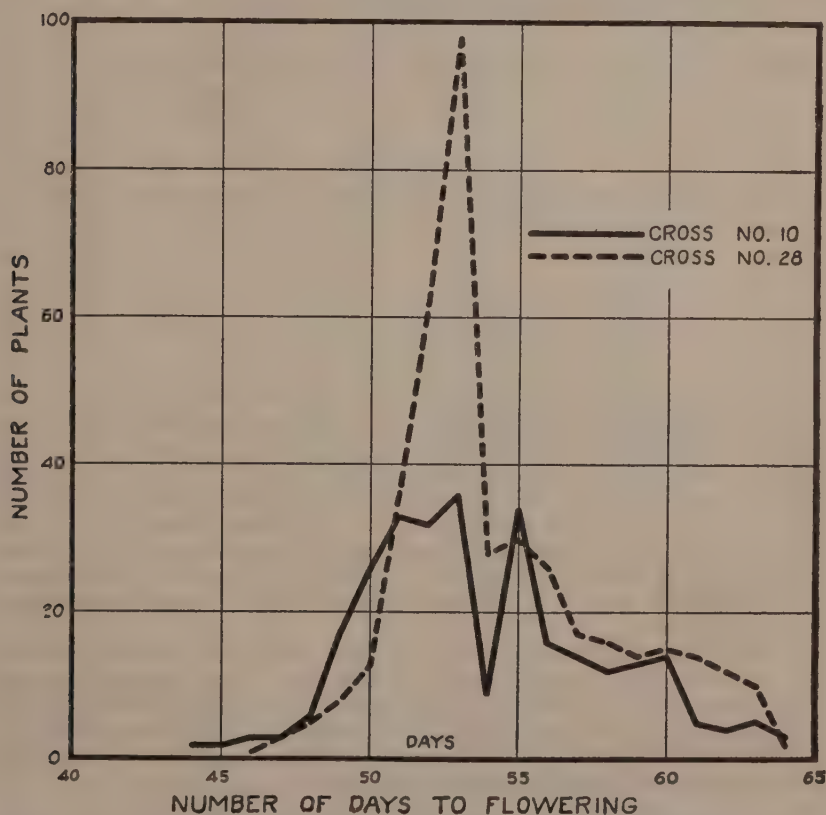


TABLE 8. Frequency distributions, means and coefficients of variation for the number of days from date of planting to flowering

Parents and hybrids	Gen-eration	Class centers in days													Means		Coefficients of variation
		45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	
Trebi Glabron Hybrid—10	P <sub>1</sub> P <sub>1</sub> P <sub>1</sub>									5	6	6					75.3 ± 0.1 71.5 ± 0.0 70.2 ± 0.1
Trebi Glabron Hybrid—10	P <sub>2</sub> P <sub>2</sub> P <sub>2</sub>				9 17 79	47 45 42	17 42 32	14 10 12									4.5 ± 0.2 58.2 ± 0.2 53.8 ± 0.2
Trebi Glabron Hybrid—10	P <sub>3</sub> P <sub>3</sub> P <sub>3</sub>	7	26	91				2	35	528	686	72	2	6			70.8 ± 0.0 73.0 ± 0.0 70.2 ± 0.0
Trebi Glabron Hybrid—10	P <sub>3</sub> P <sub>3</sub> P <sub>3</sub>					29	224	402	8	1796	860	403	61	51	1	1	2.8 ± 0.0 2.8 ± 0.0 2.8 ± 0.0
Trebi Comfort Hybrid—23	P <sub>1</sub> P <sub>1</sub> P <sub>1</sub>										8	6					75.3 ± 0.1 73.0 ± 0.2 73.1 ± 0.1
Trebi Comfort Hybrid—23	P <sub>2</sub> P <sub>2</sub> P <sub>2</sub>				27	32	31	6			7	2					57.5 ± 0.2 55.6 ± 0.1 55.5 ± 0.1
Trebi Comfort Hybrid—23	P <sub>3</sub> P <sub>3</sub> P <sub>3</sub>			24	115	85	29	7									1.2 ± 0.2 1.2 ± 0.2 1.2 ± 0.2
Trebi Velvet Hybrid—28	P <sub>1</sub> P <sub>1</sub> P <sub>1</sub>									2	2	4	2				76.0 ± 0.3 73.4 ± 0.2 71.1 ± 0.1
Trebi Velvet Hybrid—28	P <sub>2</sub> P <sub>2</sub> P <sub>2</sub>				14	26	21	13									58.3 ± 0.1 57.8 ± 0.1 54.5 ± 0.1
Trebi Velvet Hybrid—28	P <sub>3</sub> P <sub>3</sub> P <sub>3</sub>	1	16	112	156	59	43	24									72.0 ± 0.0 72.4 ± 0.1 70.6 ± 0.0
Trebi Velvet Hybrid—28	P <sub>3</sub> P <sub>3</sub> P <sub>3</sub>					1	3	3	16	188	949	169	32	8		1	2.9 ± 0.0 4.6 ± 0.1 6.4 ± 0.0
Trebi Glabron Hybrid—32	P <sub>1</sub> P <sub>1</sub> P <sub>1</sub>										3	4	2				75.7 ± 0.3 77.2 ± 0.2 71.3 ± 0.3
Trebi Glabron Hybrid—32	P <sub>2</sub> P <sub>2</sub> P <sub>2</sub>				10	18	17	16									58.9 ± 0.3 57.6 ± 0.2 53.3 ± 0.2
Trebi Glabron Hybrid—32	P <sub>3</sub> P <sub>3</sub> P <sub>3</sub>		19	66	29	61	27	15									72.5 ± 0.2 72.5 ± 0.2 70.8 ± 0.2
Trebi Glabron Hybrid—33	P <sub>1</sub> P <sub>1</sub> P <sub>1</sub>									2	3	3					73.5 ± 0.2 72.5 ± 0.2 70.8 ± 0.2
Trebi Glabron Hybrid—33	P <sub>2</sub> P <sub>2</sub> P <sub>2</sub>				12	41	36	7									58.2 ± 0.2 57.5 ± 0.1 54.2 ± 0.2
Trebi Glabron Hybrid—33	P <sub>3</sub> P <sub>3</sub> P <sub>3</sub>	1	15	98	69	30	39	13									4.1 ± 0.2 3.6 ± 0.1 7.4 ± 0.2

TABLE 9. *Frequency distributions of  $F_2$  plants according to number of days from planting to flowering using a class interval of one day*

No. days	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	Tot.
Cross 10	2	2	3	3	6	17	26	33	32	36	9	34	16	14	12	13	14	5	4	4	3	299
Cross 28			1	3	5	8	13	36	63	98	28	30	26	17	16	14	15	14	12	10	2	411

Fig. 1. Curves showing distributions of  $F_2$  plants according to number of days from date of planting to flowering.

The frequency curves show some indication of a dividing line between early and late plants at 54 days. The plants in each cross were arbitrarily divided into early and late groups using 54 days as the dividing line. The plants falling at 54 days were equally divided between the early and late

groups. Using this arbitrary division point, there are in cross No. 10, 165 plants in the early group and 124 plants in the late group. In cross No. 28 there are 241 plants in the early group and 170 plants in the late group. The numbers of early and late plants in both crosses are very close to the theoretical numbers calculated on the basis of a 9:7 ratio, as shown in table 10.

The  $F_2$  data as analyzed suggest the presence of two important allelomorphic pairs of complementary factors for earliness.

TABLE 10. *Numbers of early and late plants in the  $F_2$  generation compared with the theoretical numbers on the basis of a 9:7 ratio*

Cross	Phenotypes	No. of plants		Dev.	Dev.
		Actual	Theoretical		P.E.
No. 10	Early	165	162.56	2.44	0.42
	Late	124	126.44		
No. 28	Early	241	231.19	9.81	1.44
	Late	170	179.81		

$F_3$  generation. A further study of the inheritance of earliness was made in the third generation. Assuming two complementary factors for earliness giving an  $F_2$  ratio of nine early to seven late plants, the late  $F_2$  plants should produce late  $F_3$  progenies and the early  $F_2$  plants should produce one true breeding early  $F_3$  progeny to four progenies segregating in a 3:1 ratio to four progenies segregating in a 9:7 ratio. Because of the predominance of early plants in the segregating progenies, all the progenies of early plants would be comparatively early as judged by their means. This would cause a ratio of nine early to seven late  $F_3$  progenies as judged by their means.

Table 11 shows the distribution of the  $F_3$  families according to their mean number of days from planting to flowering. These data are graphically represented in figure 2. The curves in figure 2 show a distinct break at 71 days. Dividing the progenies into early and late groups, using 71 days as the dividing line, gives 82 early to 62 late families in cross No. 10 and 84 early to 60 late families in cross No. 28.

TABLE 11. *Distribution of  $F_3$  families according to mean number of days from planting to flowering*

No. days	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	Tot.
Cross 10	1	1	2	3	6	7	13	17	16	13	6	16	15	10	9	4	4	1	144
Cross 28				1	2	5	13	22	20	18	7	11	10	9	10	8	5	3	144

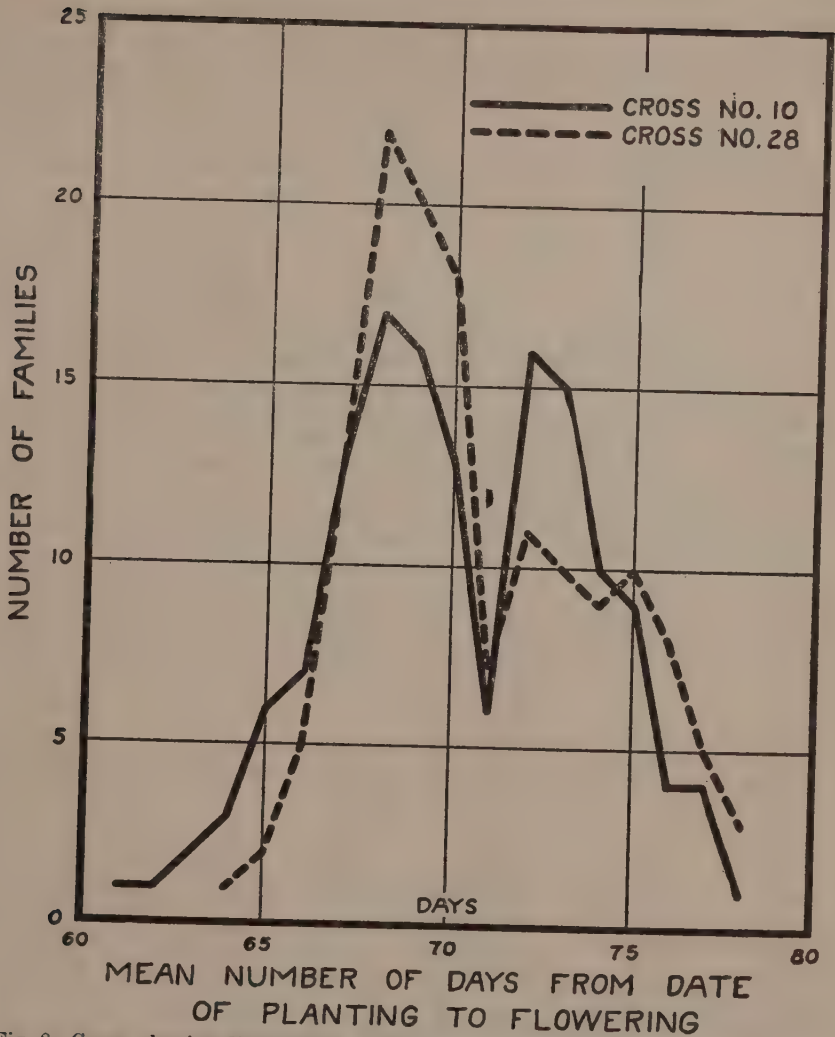


Fig. 2. Curves showing distributions of  $F_3$  families according to mean number of days from date of planting to flowering.

Table 12 shows the numbers of early and late  $F_3$  families as compared with the theoretical numbers calculated on the basis of a 9:7 ratio. The actual numbers are very near those expected theoretically.

In order to determine the breeding behavior in the individual  $F_3$  families a large table was made showing the frequency distribution of



TABLE 12. *Numbers of early and late  $F_3$  families compared with the theoretical numbers on the basis of a 9:7 ratio*

Cross	Phenotypes	No. of families		Dev.	Dev.
		Actual	Theoretical		P.E.
Cross 10	Early	82	81	1.0	0.24
	Late	62	63		
Cross 28	Early	84	81	3.0	0.74
	Late	60	63		

each  $F_3$  family according to number of days from planting to flowering. When a perpendicular line was drawn through the table at 71 days it was observed that some of the families fell completely, or nearly so, to the left of this line, while others fell to the right of the line and still others showed appreciable numbers of plants on both sides of the line. The families falling to the left of the arbitrary line were classed as true breeding early families, while those falling to the right of the line were considered true breeding late families and those having appreciable numbers of plants on each side of the line were classified as segregating families. The segregating families were then classified into 3:1 and 9:7 ratios, depending upon which of the two ratios they fitted as judged by the deviations divided by the probable errors. When the  $F_3$  families were classified in this manner the numbers shown in table 13 were obtained. These numbers are reasonably close to the numbers expected on the basis of a theoretical ratio of 1:4:4:7.

TABLE 13.  *$F_3$  families classified according to breeding behavior, and the numbers in each class compared with the theoretical numbers on the basis of the theoretical ratio of 1:4:4:7*

Description of $F_3$ families	Number of families			
	Cross no. 10		Cross no 28	
	Observed	Calculated	Observed	Calculated
Breeding true for earliness	13	9	12	9
Segregating, 3 early to 1 late	40	36	41	36
Segregating, 9 early to 7 late	39	36	41	36
Breeding true for lateness	52	63	50	63
	$X^2$	4.69	$X^2$	5.06
	P	.138	P	.169

Tables 14, 15 and 16 show the numbers of early and late plants in the individual  $F_3$  families grouped according to the way the families were

classified. It will be noted that the totals in each of the segregating groups fit closely the theoretical numbers.

The data obtained in the  $F_3$  generation seem to substantiate the hypothesis that, in the crosses studied, earliness is dominant to lateness and that two important complementary factors are involved.

TABLE 14. *Numbers of early and late plants in  $F_3$  families classified as showing 3:1 and 9:7 ratios (Cross No. 10)*

3:1 ratios			9:7 ratios				
Family no.	Early	Late	Family no.	Early	Late		
38	33	12	51	28	19		
40	38	9	56	28	17		
43	41	5	64	27	18		
44	33	12	70	23	18		
47	31	15	75	26	19		
59	32	11	79	26	17		
61	30	12	80	29	17		
63	35	10	81	22	17		
67	33	10	85	27	15		
68	31	14	86	26	16		
73	35	10	88	23	22		
76	30	11	90	25	19		
82	24	10	91	24	15		
83	31	7	92	24	22		
89	28	10	97	25	15		
94	35	9	103	27	21		
95	33	6	107	26	15		
96	32	10	113	18	18		
100	37	10	114	26	14		
102	34	11	116	21	16		
104	36	11	117	22	20		
106	39	8	119	23	20		
111	40	7	120	24	16		
112	32	5	121	21	17		
122	28	10	126	26	18		
123	30	9	129	26	21		
124	36	7	135	23	19		
127	34	10	139	24	20		
131	37	7	140	23	15		
136	31	14	141	30	19		
137	33	12	148	25	20		
142	26	10	149	25	15		
147	29	10	153	24	16		
151	28	14	154	23	23		
155	31	10	167	20	22		
156	30	13	169	23	13		
158	31	15	171	25	17		
159	30	8	176	27	19		
165	30	8	179	24	21		
173	32	5					
Total	Act. Theo.	1299 1272	397 424	Total	Act. Theo.	959 933.75	701 726.25

TABLE 15. *Numbers of early and late plants in  $F_2$  families classified as showing 3:1 and 9:7 ratios (Cross No. 28)*

3:1 ratios			9:7 ratios				
Family no.	Early	Late	Family no.	Early	Late		
256	33	6	259	36	18		
257	38	8	260	23	19		
266	31	11	262	25	16		
269	37	11	264	19	13		
274	33	11	268	22	18		
277	27	12	271	23	25		
278	31	11	280	15	9		
279	39	9	284	23	18		
283	43	7	285	21	22		
287	38	4	287	29	13		
291	42	6	288	24	19		
292	41	4	290	25	18		
295	11	5	294	24	18		
299	25	12	298	12	12		
310	36	7	305	20	19		
313	36	9	311	18	17		
319	39	6	312	28	18		
320	38	7	314	23	15		
324	26	7	315	24	16		
325	34	10	316	25	18		
326	36	9	317	26	19		
329	39	7	318	24	13		
336	27	6	322	26	14		
346	32	12	330	23	19		
347	24	7	333	28	16		
349	24	8	335	24	21		
350	32	11	339	25	16		
353	31	11	340	26	13		
356	36	4	348	22	13		
365	33	11	354	20	26		
368	35	8	355	17	15		
372	37	10	359	28	16		
373	31	12	364	28	18		
374	31	12	367	21	16		
376	24	9	375	23	16		
377	30	5	378	21	17		
379	39	8	379	14	22		
380	33	14	384	24	20		
391	29	12	385	21	18		
394	35	8	387	25	20		
395	35	10	396	26	21		
Total	Act. Theo.	1351 1281	357 427	Total	Act. Theo.	951 934.3	710 726.7

TABLE 16. *Numbers of early and late plants in F<sub>2</sub> families classified as breeding true for earliness and for lateness (Crosses No. 10 and 28)*

Breeding true for lateness				Breeding true for earliness			
Family no.	Early	Late		Family no.	Early	Late	
X10				X38			
39	1	46	34	297	1	38	
41	1	44	35	300	1	41	
42	0	40	28	301	2	26	
45	1	39	0	302	2	38	
46	1	45	37	303	1	45	
48	1	40	161	306	6	36	
49	1	44	162	307	0	40	
53	5	40	163	308	0	87	
54	1	47	164	309	4	43	
57	2	33	166	321	0	32	
58	2	45	168	323	1	41	
60	2	39	170	327	1	31	
62	1	39	172	328	1	43	
65	2	45	175	331	1	39	
69	0	45	177	332	0	39	
71	1	37	178	334	1	46	
72	1	40	180	337	1	42	
74	2	37	253	338	5	32	
78	0	44	254	341	5	34	
84	2	36	255	352	1	38	
98	1	34	258	357	0	44	
99	3	33	261	358	2	40	
101	1	41	263	360	3	30	
105	3	39	265	361	1	38	
108	1	40	267	362	0	40	
118	1	42	270	363	0	44	
125	2	40	272	371	1	43	
128	2	39	273	382	1	42	
130	2	38	275	383	4	28	
132	1	43	276	386	1	39	
133	2	43	281	389	9	33	
138	1	39	282	392	1	43	
143	0	38	286	393	3	40	
144	1	37	293				
145	0	34	296				
X28				X28			
304	1	44	304	304	1	44	
342	1	46	342	342	1	46	
343	1	45	343	343	1	45	
344	3	34	344	344	3	34	
351	2	31	351	351	2	31	
43	0	43	43	43	0	43	
44	0	44	44	44	0	44	
369	37	37	369	369	37	37	
370	40	30	370	370	40	30	
381	40	38	381	381	40	38	
40	0	40	40	40	0	40	
386	40	44	386	386	40	44	
390	44	44	390	390	44	44	



TABLE 17. *Means and coefficients of variation for plant height*

Parents and hybrids	Generation	Number of plants	Means	Coefficients of variation
Trebi	P <sub>1</sub>	6	113.3 ± 1.5	4.5 ± 1.0
Glabron	P <sub>1</sub>	6	135.0 ± 2.3	5.7 ± 1.2
Hybrid—10	F <sub>1</sub>	6	131.7 ± 1.5	3.9 ± 0.8
Trebi	P <sub>2</sub>	96	64.0 ± 0.4	8.4 ± 0.4
Glabron	P <sub>2</sub>	135	76.1 ± 0.3	6.8 ± 0.3
Hybrid—10	F <sub>2</sub>	299	68.0 ± 0.3	10.6 ± 0.3
Trebi	P <sub>3</sub>	1325	78.0 ± 0.1	9.5 ± 0.1
Glabron	P <sub>3</sub>	1399	94.6 ± 0.1	7.9 ± 0.1
Hybrid—10	F <sub>3</sub>	6042	85.9 ± 0.1	11.1 ± 0.1
Trebi	P <sub>1</sub>	6	106.7 ± 0.8	2.4 ± 0.5
Comfort	P <sub>1</sub>	12	123.3 ± 1.5	5.8 ± 0.8
Hybrid—23	F <sub>1</sub>	9	128.9 ± 1.0	3.2 ± 0.5
Trebi	P <sub>2</sub>	107	59.4 ± 0.2	7.5 ± 0.3
Comfort	P <sub>2</sub>	278	64.9 ± 0.4	10.0 ± 0.3
Hybrid—23	F <sub>2</sub>	367	65.4 ± 0.2	10.5 ± 0.3
Trebi	P <sub>1</sub>	6	116.7 ± 2.3	6.4 ± 1.4
Velvet	P <sub>1</sub>	12	151.2 ± 0.8	2.5 ± 0.4
Hybrid—28	F <sub>1</sub>	9	139.4 ± 0.7	2.0 ± 0.3
Trebi	P <sub>3</sub>	79	66.1 ± 0.4	8.2 ± 0.4
Velvet	P <sub>3</sub>	309	87.1 ± 0.2	7.3 ± 0.2
Hybrid—28	F <sub>2</sub>	455	72.5 ± 0.2	11.2 ± 0.2
Trebi	P <sub>3</sub>	1368	76.0 ± 0.1	8.6 ± 0.1
Velvet	P <sub>3</sub>	1433	109.3 ± 0.2	9.0 ± 0.1
Hybrid—28	F <sub>3</sub>	5816	88.8 ± 0.1	10.7 ± 0.1
Trebi	P <sub>1</sub>	6	114.1 ± 1.1	3.3 ± 0.7
Glabron	P <sub>1</sub>	6	123.3 ± 1.5	4.2 ± 0.9
Hybrid—32	F <sub>1</sub>	3	126.7 ± 2.8	4.5 ± 1.5
Trebi	P <sub>2</sub>	69	67.4 ± 0.4	7.7 ± 0.4
Glabron	P <sub>2</sub>	163	75.1 ± 0.2	6.2 ± 0.2
Hybrid—32	F <sub>2</sub>	167	69.9 ± 0.4	10.4 ± 0.4
Trebi	P <sub>1</sub>	6	114.2 ± 1.8	5.1 ± 1.1
Glabron	P <sub>1</sub>	6	140.0 ± 2.1	5.0 ± 1.1
Hybrid—33	F <sub>1</sub>	6	137.5 ± 0.8	2.0 ± 0.4
Trebi	P <sub>2</sub>	103	69.0 ± 0.3	6.8 ± 0.2
Glabron	P <sub>2</sub>	167	83.8 ± 0.3	6.0 ± 0.2
Hybrid—33	F <sub>2</sub>	283	74.9 ± 0.3	10.1 ± 0.3

## HEIGHT OF PLANTS

The mean heights and coefficients of variation for the F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> progenies and their parents in the five crosses studied are given in table 17.

The male and female parents in each cross are significantly different in their means for height. Trebi, the male parent, is shorter than the female parent in each case.

As shown by the means for height, the F<sub>1</sub> hybrid progenies are invariably taller than their male parents and approach their female parents. The differences between the F<sub>2</sub> progenies and their male parents are statistically significant. They are more than six times the size of their probable errors. In the F<sub>3</sub> populations the differences are more than forty times their probable errors.

The coefficients of variation show that in the F<sub>2</sub> and F<sub>3</sub> generations plant height is considerably more variable than in either of the parents grown the same year. This is an indication of segregation of genetic factors for height of plant.

TABLE 18. Means and coefficients of variation for number of culms

Parents and hybrids	Generation	Number of plants	Means	Coefficients of variation
Trebi	P <sub>1</sub>	6	4.5 ± 0.6	46.0 ± 9.8
Glabron	P <sub>1</sub>	6	4.5 ± 0.3	24.7 ± 5.3
Hybrid—10	F <sub>1</sub>	6	4.8 ± 0.0	30.9 ± 6.6
Trebi	P <sub>2</sub>	96	7.6 ± 0.2	40.5 ± 1.5
Glabron	P <sub>2</sub>	135	9.3 ± 0.2	38.9 ± 1.6
Hybrid—10	F <sub>2</sub>	299	7.3 ± 0.1	44.1 ± 1.2
Trebi	P <sub>3</sub>	1325	7.5 ± 0.1	39.4 ± 0.5
Glabron	P <sub>3</sub>	1398	8.6 ± 0.1	36.7 ± 0.5
Hybrid—10	F <sub>3</sub>	6042	7.5 ± 0.0	39.8 ± 0.2
Trebi	P <sub>1</sub>	6	5.0 ± 0.5	34.8 ± 7.4
Comfort	P <sub>1</sub>	12	6.7 ± 0.3	21.8 ± 3.1
Hybrid—23	F <sub>1</sub>	9	5.0 ± 0.5	40.2 ± 6.8
Trebi	P <sub>2</sub>	107	7.1 ± 0.2	49.0 ± 2.3
Comfort	P <sub>2</sub>	278	8.7 ± 0.1	43.4 ± 1.2
Hybrid—23	F <sub>2</sub>	367	8.2 ± 0.1	44.8 ± 0.7
Trebi	P <sub>3</sub>	6	4.0 ± 0.5	35.2 ± 7.5
Velvet	P <sub>1</sub>	12	4.5 ± 0.3	36.7 ± 5.3
Hybrid—28	F <sub>1</sub>	9	2.7 ± 0.3	47.2 ± 8.0
Trebi	P <sub>2</sub>	79	8.8 ± 0.3	44.8 ± 1.6
Velvet	P <sub>2</sub>	309	6.7 ± 0.1	37.8 ± 1.0
Hybrid—28	F <sub>2</sub>	455	6.4 ± 0.1	44.7 ± 0.7
Trebi	P <sub>3</sub>	1368	7.0 ± 0.0	40.3 ± 0.5
Velvet	P <sub>3</sub>	1433	6.7 ± 0.0	43.6 ± 0.5
Hybrid—28	F <sub>3</sub>	5816	6.3 ± 0.0	46.1 ± 0.0
Trebi	P <sub>1</sub>	6	5.5 ± 0.3	20.2 ± 4.3
Glabron	P <sub>1</sub>	6	5.5 ± 0.3	20.2 ± 4.3
Hybrid—32	F <sub>1</sub>	3	5.7 ± 0.3	10.2 ± 3.4
Trebi	P <sub>2</sub>	69	9.2 ± 0.4	49.7 ± 2.8
Glabron	P <sub>2</sub>	163	10.5 ± 0.2	37.8 ± 1.4
Hybrid—32	F <sub>2</sub>	167	8.9 ± 0.2	42.6 ± 1.6
Trebi	P <sub>3</sub>	6	4.5 ± 0.6	46.0 ± 9.8
Glabron	P <sub>3</sub>	6	2.5 ± 0.3	44.4 ± 9.5
Hybrid—33	F <sub>1</sub>	6	4.5 ± 0.3	24.7 ± 5.3
Trebi	P <sub>2</sub>	103	7.8 ± 0.1	38.1 ± 1.8
Glabron	P <sub>2</sub>	167	7.8 ± 0.1	38.1 ± 1.4
Hybrid—33	F <sub>2</sub>	283	8.5 ± 0.1	44.0 ± 1.2

That height of plant is inherited in the Mendelian fashion exhibited by qualitative characters is indicated by the facts that the F<sub>1</sub> hybrids are as uniform as their parents, that the variability in height is much greater in the second than in the first hybrid generation, presumably because of new combinations of factors, and that both of the parental types are recovered in the hybrid generations. Plants as short as the shortest plants in the short parent and as tall as the tallest plants in the tall parent were obtained in the F<sub>2</sub> generation in all cases excepting in cross No. 10. In this case the extreme parental forms were recovered in the F<sub>3</sub> generation.

One cannot help but notice the great difference in height of plants when comparing the three generations. It is evident that the generation grown in the green house (F<sub>1</sub> and parents) had a strong tendency toward vegetative growth even though the winter and early spring days were lengthened by use of artificial light. The difference between the F<sub>2</sub> and F<sub>3</sub> generations is probably largely due to the difference in dates of planting. The F<sub>2</sub> generation was planted in the field on April 27, while the F<sub>3</sub> gen-

eration was planted on April 5. Height of plant is a character which is greatly influenced by environmental factors.

#### NUMBER OF CULMS

Table 18, which presents means and coefficients of variation for number of culms, does not offer much evidence of the inheritance of this character. It may be that the character is so much influenced by environment that genetic influences are largely overshadowed. Harlan (9) found the number of culms to be greatly influenced by environment. It is interesting to note the small number of culms in the first generation as compared to the other generations. The first generation was grown in the greenhouse. Apparently the barley plants did not stool as much under the greenhouse conditions as they did in the field.

Cross No. 33 showed a significant difference between the two parents for number of culms in the first generation, but the next year the difference was much smaller. The coefficient of variation was somewhat larger in the  $F_2$  hybrid population than in the parental populations. There is some slight evidence of the action of genetic factors for number of culms in this cross.

#### LENGTH OF SPIKE

Trebi, the male parent in each cross, had shorter spikes than the varieties used as female parents. In table 19, it is seen that the  $F_1$  hybrid plants had spikes that were longer than those of Trebi, but approximately equal to those of the female parent in each case, indicating dominance of long spikes.

Cross No. 23 seems to differ from the other four crosses in that the two parents are practically equal in length of spike. That the two parents in this cross are similar in their genetic factors for length of spike is indicated by the lack of increased variability of the  $F_2$  hybrid population over the variability of the  $F_1$  and parental populations. In the other crosses the variability of the  $F_2$  and  $F_3$  populations shows evidence of the segregation of genetic factors.

#### PLANT YIELD

An examination of table 20 rather leads one to believe that the parents used in the crosses did not differ much in their genetic makeup for yield. Crosses No. 23, No. 28 and No. 33 exhibit in the first generation, differences in favor of the Trebi parent which border on statistical significance. In the cross No. 23, however, which shows the greatest difference in favor of the Trebi parents in the first generation, there is a slight difference in favor of the female parent the next year. In cross No. 32 the first generation shows a slight difference in favor of the female parent and the second generation a slight difference in favor of the Trebi parent.

Additional indication that the parents did not differ significantly in genetic factors for yield is the fact that the coefficients of variation in the  $F_2$  hybrid populations are no larger than those of their parents. In fact, the coefficients of variation in this generation are, in four of the crosses, slightly smaller than those of either parent. The frequency distributions

TABLE 19. *Means and coefficients of variation for length of average spike*

Parents and hybrids	Generation	Number of plants	Means	Coefficients of variation
Trebi	P <sub>1</sub>	6	6.9 ± 0.1	6.1 ± 1.3
Glabron	P <sub>1</sub>	6	8.1 ± 0.2	6.4 ± 1.4
Hybrid—10	F <sub>1</sub>	6	8.1 ± 0.2	6.4 ± 1.4
Trebi	P <sub>2</sub>	96	7.1 ± 0.1	11.3 ± 0.5
Glabron	P <sub>2</sub>	135	8.5 ± 0.1	12.0 ± 0.5
Hybrid—10	F <sub>2</sub>	299	7.7 ± 0.0	13.7 ± 0.4
Trebi	P <sub>3</sub>	1325	7.0 ± 0.0	11.2 ± 0.1
Glabron	P <sub>3</sub>	1399	7.9 ± 0.0	12.0 ± 0.0
Hybrid—10	F <sub>3</sub>	6042	7.0 ± 0.0	15.6 ± 0.1
Trebi	P <sub>1</sub>	6	7.2 ± 0.2	7.6 ± 1.6
Comfort	P <sub>1</sub>	12	7.4 ± 0.2	13.2 ± 1.9
Hybrid—23	F <sub>1</sub>	9	8.0 ± 0.2	8.4 ± 1.4
Trebi	P <sub>2</sub>	107	7.5 ± 0.0	10.1 ± 0.5
Comfort	P <sub>2</sub>	278	7.3 ± 0.0	12.5 ± 0.4
Hybrid—23	F <sub>2</sub>	367	7.8 ± 0.0	10.6 ± 0.3
Trebi	P <sub>1</sub>	6	6.6 ± 0.1	6.2 ± 1.3
Velvet	P <sub>1</sub>	12	8.8 ± 0.1	7.6 ± 1.1
Hybrid—28	F <sub>1</sub>	9	8.4 ± 0.2	8.4 ± 1.4
Trebi	P <sub>2</sub>	79	7.7 ± 0.1	9.7 ± 0.5
Velvet	P <sub>2</sub>	309	8.9 ± 0.0	11.0 ± 0.3
Hybrid—28	F <sub>2</sub>	455	7.8 ± 0.0	13.6 ± 0.3
Trebi	P <sub>3</sub>	1368	7.0 ± 0.0	12.8 ± 0.2
Velvet	P <sub>3</sub>	1433	8.2 ± 0.0	12.5 ± 0.2
Hybrid—28	F <sub>3</sub>	5816	7.3 ± 0.0	14.4 ± 0.0
Trebi	P <sub>1</sub>	6	6.7 ± 0.2	9.3 ± 2.0
Glabron	P <sub>1</sub>	6	7.9 ± 0.2	8.2 ± 1.7
Hybrid—32	F <sub>1</sub>	3	8.1 ± 0.3	7.2 ± 2.4
Trebi	P <sub>2</sub>	69	7.7 ± 0.1	10.6 ± 0.6
Glabron	P <sub>2</sub>	163	8.3 ± 0.0	11.1 ± 0.4
Hybrid—32	F <sub>2</sub>	167	8.7 ± 0.1	13.9 ± 0.5
Trebi	P <sub>1</sub>	6	6.7 ± 0.2	9.3 ± 2.0
Glabron	P <sub>1</sub>	6	7.7 ± 0.3	11.5 ± 2.4
Hybrid—33	F <sub>1</sub>	6	8.6 ± 0.1	4.8 ± 1.0
Trebi	P <sub>2</sub>	103	7.4 ± 0.1	11.5 ± 0.5
Glabron	P <sub>2</sub>	167	7.7 ± 0.0	12.6 ± 0.5
Hybrid—33	F <sub>2</sub>	283	7.6 ± 0.0	12.9 ± 0.4

of parents and hybrids are strikingly similar in each generation of each cross.

The data in table 20 raise the question as to the possibilities of selection for yield in these crosses. It may be that the genetic differences in yield were covered up by environmental conditions and that selection for yield over a period of years in the hybrid populations actually would be effective in isolating high yielding strains.

#### CORRELATION STUDIES

In these studies, correlations between plant yield and other quantitative characters in the F<sub>2</sub> generation were calculated. In the F<sub>3</sub> generation, correlation coefficients were calculated for all possible combinations of the characters under consideration. Finally, parent-progeny correlations were calculated with the hope of obtaining some idea of the extent to which such quantitative characters as studied in the selected F<sub>2</sub> plants were expressed in their F<sub>3</sub> progenies.



TABLE 20. *Means and coefficients of variation for plant yield*

Parents and hybrids	Generation	Number of plants	Means	Coefficients of variation
Trebi	P <sub>1</sub>	6	5.2 ± 0.2	15.9 ± 3.4
Glabron	P <sub>1</sub>	6	4.8 ± 0.3	21.3 ± 4.5
Hybrid—10	F <sub>1</sub>	6	5.8 ± 0.2	14.1 ± 3.0
Trebi	P <sub>2</sub>	96	8.5 ± 0.3	53.9 ± 2.6
Glabron	P <sub>2</sub>	135	9.5 ± 0.3	47.3 ± 1.9
Hybrid—10	F <sub>2</sub>	299	9.8 ± 0.2	46.8 ± 1.3
Trebi	P <sub>3</sub>	1325	11.1 ± 0.1	44.8 ± 0.6
Glabron	P <sub>3</sub>	1399	10.7 ± 0.1	44.2 ± 0.6
Hybrid—10	F <sub>3</sub>	6042	10.6 ± 0.0	46.7 ± 0.3
Trebi	P <sub>1</sub>	6	3.8 ± 0.4	39.2 ± 8.4
Comfort	P <sub>1</sub>	12	2.5 ± 0.2	41.6 ± 6.0
Hybrid—23	F <sub>1</sub>	9	4.8 ± 0.5	41.4 ± 7.0
Trebi	P <sub>2</sub>	107	7.7 ± 0.3	59.7 ± 2.7
Comfort	P <sub>2</sub>	278	7.9 ± 0.2	57.2 ± 1.6
Hybrid—23	F <sub>2</sub>	367	10.6 ± 0.2	56.4 ± 1.4
Trebi	P <sub>1</sub>	6	6.2 ± 0.4	24.5 ± 5.2
Velvet	P <sub>1</sub>	12	4.8 ± 0.2	20.3 ± 2.9
Hybrid—28	F <sub>1</sub>	9	5.3 ± 0.2	12.7 ± 2.1
Trebi	P <sub>2</sub>	79	10.1 ± 0.4	55.7 ± 2.9
Velvet	P <sub>2</sub>	309	8.1 ± 0.2	52.8 ± 1.4
Hybrid—28	F <sub>2</sub>	455	10.0 ± 0.2	49.6 ± 1.1
Trebi	P <sub>3</sub>	1368	9.6 ± 0.1	50.4 ± 0.6
Velvet	P <sub>3</sub>	1433	10.2 ± 0.0	26.7 ± 0.3
Hybrid—28	F <sub>3</sub>	5816	9.6 ± 0.0	54.8 ± 0.0
Trebi	P <sub>1</sub>	6	4.8 ± 0.5	33.7 ± 7.2
Glabron	P <sub>1</sub>	6	5.2 ± 0.7	45.2 ± 9.6
Hybrid—32	F <sub>1</sub>	3	4.8 ± 0.5	23.8 ± 8.0
Trebi	P <sub>2</sub>	69	11.1 ± 0.5	55.2 ± 3.2
Glabron	P <sub>2</sub>	163	10.1 ± 0.2	40.6 ± 1.5
Hybrid—32	F <sub>2</sub>	167	12.7 ± 0.3	47.2 ± 1.7
Trebi	P <sub>1</sub>	6	4.8 ± 0.3	21.3 ± 4.5
Glabron	P <sub>1</sub>	6	3.8 ± 0.4	39.2 ± 8.4
Hybrid—33	F <sub>1</sub>	6	5.2 ± 0.4	25.0 ± 5.3
Trebi	P <sub>2</sub>	103	10.1 ± 0.3	48.6 ± 2.3
Glabron	P <sub>2</sub>	167	8.0 ± 0.2	53.2 ± 2.0
Hybrid	F <sub>2</sub>	283	12.5 ± 0.2	49.3 ± 1.4

*Correlation Between Yield and the Other Characters in the F<sub>2</sub> Generation.* Table 21 shows the correlation coefficients between yield and each of four other characters in four parents and five crosses. For crosses No. 10 and No. 28, these correlation coefficients include both the parental strains and their hybrid progenies. This should give some evidence as to whether the correlations found in the hybrid populations are caused by the association or linkage of genetic factors or whether they are caused by physiological relationships. If the correlation coefficients in the hybrid populations do not significantly differ from those of the parental strains one could hardly say that the correlations were caused by the association of genetic factors, but would have to be attributed to physiological causes.

In table 21 there is a strikingly high correlation between yield and number of culms. Evidently number of culms was an important factor in determining yield. This assumption is verified by a study of the beta regression coefficients in a later paragraph. The other three characters studied seem to have an appreciable influence on yield. There is a negative corre-

lation between yield and number of days to flowering. In other words, there is in these plants an association between high yield and early flowering.

TABLE 21. *Coefficients of correlation between yield and the other four characters studied in the  $F_2$  generation*

Parents and hybrids	Length of spike		Number of culms		Height		Days to flowering	
	n	r	n	r	n	r	n	r
Trebi, M10	96	0.39	96	0.80	96	0.42	87	—0.44
Glabron, F10	135	0.41	135	0.80	135	0.41	114	—0.26
Glabron x Trebi, X10	299	0.37	299	0.80	299	0.49	289	—0.22
Trebi, M10	79	0.27	79	0.84	79	0.30	74	—0.55
Velvet, F10	309	0.39	309	0.71	309	0.43	262	—0.18
Velvet x Trebi, X28	455	0.37	455	0.78	455	0.47	411	—0.10
Comfort x Trebi, X23	367	0.41	367	0.87	367	0.58	345	—0.50
Glabron x Trebi, X32	167	0.28	167	0.78	167	0.52	149	—0.12
Glabron x Trebi, X33	283	0.47	283	0.81	283	0.47	265	—0.12

Note: These coefficients of correlation are significant as tested by Fisher's method, excepting those printed in italics.

It will be noted that there are no consistent differences between the correlation coefficients in the hybrid population in the parental strains, indicating that the associations are physiologic rather than genetic, or that there are no major differences in the genotypes for yield in the parents.

*Simple Correlation Between Characters in  $F_3$  Generation.* Table 22 shows correlation coefficients calculated for all possible combinations of characters in the  $F_3$  generation. The correlations between yield and other characters check very closely with the same correlations obtained in the

TABLE 22. *Correlation of characters in the  $F_3$  generation*

Characters	Correlation coefficients				n
	Length of spike	Number of culms	Height	No. of days to flowering	
Cross No. 10					
Yield	0.37	0.83	0.33	—0.22	6,042
Length of spike		0.29	0.39	0.13	
Number of culms			0.21	—0.12	
Height				—0.08	
Cross No. 23					
Yield	0.45	0.80	0.43	—0.27	5,916
Length of spike		0.40	0.44	—0.02	
Number of culms			0.32	—0.06	
Height				—0.18	

Note: These correlation coefficients are significant as tested by Fisher's method, excepting the one printed in italics.

F<sub>2</sub> generation. The lowest correlations are those between number of days to flowering and other characters. In general, yield seems to be affected to some extent by each of the other characters, and, excepting days to flowering, all the characters seem to be significantly associated with each other.

*Multiple correlations.* In order to obtain more information on the relations between yield and the other characters studied in the F<sub>3</sub> generation, a further study was made of these sets of correlation coefficients. In this study the method of multiple regression was used for determining the relative importance of the several characters in determining yield, and for estimating the combined influence of the four characters on yield.

Following the directions of Wallace and Snedecor (26) for solving normal equations, the beta regression coefficients and the multiple correlation coefficients were calculated. The independent variables were, length of average spike (B), number of culms (C), height of plant (D), and number of days to flowering (E), and the dependent variable was yield (X). The results are given in table 23.

An interesting observation regarding the data presented in table 23 is that the two crosses gave almost the same value for the multiple correlation, R. Substituting the R value for cross No. 10 in the formula  $100 \times (1 - \sqrt{1 - R^2})$ , a value of 48.56 per cent is obtained, indicating the extent to which the total variation in yield is associated with variation in the characters studied. Applying the same formula to measure the extent to which the total variation in yielding ability of the cross No. 38 is associated with variation in the four characters under consideration, a value of 47.81 per cent is obtained. In both crosses there is still more than half of the total variation in yield which has not been accounted for.

TABLE 23. *Multiple and simple correlation coefficients and  $\beta$ -regression coefficients between yield (X) and the related characters, F<sub>3</sub> generation*

	Length of spike (B)	Number of culms (C)	Height of plant (D)	Number of days to flowering (E)
Constant				
Cross No. 10				
Correlation coefficients, r	0.37	0.83	0.33	—0.22
Regression coefficient, $\beta$	0.13	0.75	0.10	—0.14
Multiple correlation coefficient, R				0.86
Cross No. 28				
Correlation coefficients, r	0.45	0.81	0.43	—0.27
Regression coefficients, $\beta$	0.10	0.70	0.14	—0.21
Multiple correlation coefficient, R				0.85

The  $\beta$  regression coefficients substantiate the conclusion drawn from the simple correlation coefficients, that the number of culms was most closely associated with yield. The  $\beta$  regression coefficients indicate the number of days to flowering to be slightly more important than did the

simple correlation coefficients, but the other characters are of about the same relative importance. In this case, the simple correlation coefficients seem to have been reliable criteria of correlation as checked by the  $\beta$  regression coefficients.

*Parent-progeny correlations.* The plant breeder's greatest interest lies in relationships between generations. He is most interested in knowing to what extent characters are transmitted from one generation to another. Parent-progeny correlations were calculated between the  $F_2$  and  $F_3$  generations for each of the five characters. The results are presented in table 24.

TABLE 24. *Parent-progeny correlations between  $F_2$  and  $F_3$  generations for each of the five characters*

Characters	Correlation coefficients	
	Cross No. 10	Cross No. 28
Number of days to flowering	0.61	0.63
Height of the plant	0.38	0.30
Number of culms	<i>0.08</i>	<i>0.09</i>
Length of average spike	0.28	0.19
Plant yield	-0.06	-0.21

Note: These correlation coefficients are significant as tested by Fisher's method, excepting those printed in italics.  $n = 144$ .

The highest correlation coefficients in the two crosses were found for the number of days to flowering. In plant height there is a correlation between the  $F_2$  plants and their  $F_3$  progenies, but not as large as the correlation found for the number of days to flowering. Of the other six correlations, four are non-significant and the other two are small. These low parent-progeny correlations indicate that the corresponding characters are influenced more by the environmental or unknown factors than by the genetic constitution of their  $F_2$  progenitors.

#### SUMMARY AND CONCLUSIONS

1. Data are presented from five barley crosses in which the rough-awned variety, Trebi, was used as the male parent and the three smooth-awned varieties, Comfort, Glabron and Velvet, were used as female parents.
2. The characters studied were roughness of awns, time of flowering, height of plant, number of culms, length of spike and plant yield.
3. In the  $F_1$  and  $F_2$  generations, rough awns seemed to be dominant to smooth awns and the  $F_2$  data fit reasonably well a 3:1 ratio, indicating that the roughness of awns, as classified in this study, was controlled by a single pair of genetic factors. The  $F_3$  progeny tests, however, showed that the  $F_2$  segregations could not have been due to a single pair of factors. A hypothesis was presented assuming a dominant factor for smoothness of awns with a dominant inhibitor. The  $F_2$  data fit the expected 13:3 ratio almost as well as a 3:1 ratio and the  $F_3$  progeny ratios fit the theoretical expectancy very closely. The ratios of rough to smooth-awned plants in



the segregating  $F_3$  families did not fit the theoretical 13:3 ratios as closely as they should have. There probably were present modifying factors which affected the ratios in both the  $F_2$  and  $F_3$  generations.

4. In four of the five crosses the  $F_1$  and  $F_2$  generations flowered earlier than either of their parents, indicating dominance of earliness. In each of these crosses the  $F_2$  and  $F_3$  generations ranged in date of flowering from the earliest plants of the early parent to the latest plants of the late parent. A ratio of nine early to seven late plants was obtained in the  $F_2$  generation. The  $F_2$  data together with  $F_3$  progeny tests, indicated the presence of two important complementary factors for earliness.

5. The  $F_1$  hybrid population in each cross was taller than the short parent and approached closely the height of the tall parent. The  $F_1$  hybrids were as uniform as their parents, the  $F_2$  hybrids were more variable than the  $F_1$  hybrids and in each cross the parental forms were recovered in the  $F_2$  and  $F_3$  generations.

6. There was not much evidence of the inheritance of number of culms. This character seemed to be greatly influenced by environmental conditions.

7. In four of the five crosses, Trebi, the male parent, had shorter spikes than the female parent. The  $F_1$  hybrids had spikes that were longer than those of the Trebi parent and approximately equal to the female parent. The variability of the  $F_2$  and  $F_3$  hybrid generations indicated the segregation of genetic factors for length of spike. In the other cross the two parents did not differ in length of spike.

8. There was no good evidence of the segregation of genetic factors for yield in any of the five crosses.

9. In the  $F_2$  populations there were high positive correlations between number of culms and yield, and statistically significant positive correlations between height of plant and yield and length of spike and yield. The correlations between days to flowering and yield were all negative and some of them were statistically significant, meaning that there was a tendency for the earlier flowering plants to yield higher than the later flowering plants.

10. Simple and multiple correlations in the  $F_3$  populations indicated that each of the characters studied had some influence on yield.

11. High positive parent-progeny correlations were obtained for the number of days to flowering. Positive parent-progeny correlations were obtained for height of plant, but not as large as the correlations found for the number of days to flowering. The low parent-progeny correlations for height of plant, number of culms, length of average spike, and plant yield indicate that these characters are influenced more by environment than by genetic factors.

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# THE ELECTRONIC INTERPRETATION OF REACTIONS APPLIED TO HALOGEN ORGANIC COMPOUNDS<sup>1</sup>

NATHANIEL JOHN BEABER

*From the Chemical Laboratory of Iowa State College*

Accepted for publication March 18, 1931

Recently Fry<sup>2</sup> proposed an electronic formula for benzene, based on Kekulé's<sup>3</sup> original model, in which he postulates that substituents are alternately positive and negative. Thus, radicals in the 1, 2-, 1, 4- and 1, 6- positions are of opposite signs, while those in the 1, 3- and 1, 5- positions are of like sign.

In this laboratory, the Grignard reagent has been used successfully to prove the mechanism of certain reactions. It was accordingly thought worth while to test Fry's electronic formula for polyhalogen benzenes by ascertaining which would form the Grignard reagent.

It is a well established fact that in certain aryl halides the halogen carries a positive charge, while in others the halogen is negative<sup>4</sup>.

The preparation of phenylmagnesium bromide may be written electronically as follows:



From a consideration of the reactions of RMgX with various reagents, such as oxygen, carbon dioxide, aryl and alkyl halides and dimethyl sulfate, reaction (2) is universally accepted as representing the electronic structure of Grignard reagents. Thus, we have a reduction of the participating carbon, and, according to Fry's theory, this would necessitate a shift in the sign of the other carbons of the ring as well as that of radicals attached to them. According to this deduction one should be able to prepare di-Grignard reagents from 1, 2, and 1, 4-dibromobenzene and 1, 2- and 1, 4-diiodobenzene, but only a mono-Grignard reagent from 1, 3-dibromo- and 1, 3-diiodobenzene.

The unsuccessful attempts of Bodroux<sup>5</sup> to prepare a di-Grignard reagent from aryl dihalides, were substantiated by others.

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<sup>1</sup>An abstract of a thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup>Fry, The electronic conception of valence and the constitution of benzene. Longmans, Green and Co., 1921, New York, N. Y.

<sup>3</sup>Kekulé, *Ann.*, 137:129 (1866).

<sup>4</sup>Leading references on negative halogen are to be found in an article by Holleman and Heinckin, *Rec. trav. chim.*, 34, 204 (1915); for positive halogens see Nicolet, *J. Am. Chem. Soc.*, 43, 2081 (1921).

<sup>5</sup>Bodroux, *Compt. rend.* 136:1138 (1903); 137:710 (1903); *Bull. soc. chim.*, 31:24, 33 (1904).

Houben<sup>6a</sup> reported a 1.25 per cent yield of terephthalic acid from 1, 4-dibromobenzene. According to a later communication<sup>6b</sup>, when the Grignard reagent, prepared from 1, 4-dibromobenzene and magnesium, was treated with carbon disulfide, he obtained no evidence that the second halogen had reacted with magnesium.

With 1, 4-diiodobenzene, Votocek and Kohler<sup>7</sup> demonstrated for the first time that more than one halogen in aryl dihalides reacts to form the Grignard reagent. Recently, Thomas<sup>8</sup> has taken up a quantitative study of diodo derivatives of benzene and thiophene and found that 78-88 per cent of two equivalents of magnesium reacted.

Lewis and Dufford<sup>9</sup>, and Dufford and co-workers<sup>10</sup>, investigated a number of polyhalogen aryl compounds in their study of luminescence of Grignard reagents, but this work is of little value because many of their conclusions are based on erroneous assumptions.

Since Gilman and McCracken<sup>11</sup> have shown that aryl bromides in general give better yields of the Grignard reagent than the corresponding iodides, most of this work has centered around trying to find a catalyst or conditions which would cause the second halogen to react.

#### PROCEDURE

The copper-magnesium alloys used in this work were described by Gilman and co-workers<sup>12</sup>.

The reaction was carried out in the customary three-necked flask fitted with a Hopkins condenser, a mercury sealed stirrer, and a dropping funnel. A small amount of the halide was added to one equivalent of ordinary magnesium turnings and heated to boiling. If reaction did not take place on adding a small amount of ether, a drop of bromine was usually sufficient to induce reaction, after which the remainder of the halide, dissolved in four to five volumes of dry ether, was added dropwise. When the reaction had gone to completion for one equivalent of magnesium, from one-half to one equivalent of magnesium in the form of a 2 per cent or 12.5 per cent copper-magnesium alloy was added. This was followed by several hours digestion, after which the resulting Grignard reagents were decomposed with carbon dioxide.

#### EXPERIMENTAL

Since Houben<sup>5</sup> reported a .25 per cent yield of terephthalic acid from 1, 4-dibromobenzene, magnesium and carbon dioxide, a fairly large number of runs were made with this polyhalogen in which the quantity varied from 0.1 mole to 1.0 mole. In all runs one equivalent of ordinary magnesium

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<sup>6</sup>Houben, (a) *Ber.*, **38**:3796 (1905); (b) **39**:3219 (1906).

<sup>7</sup>Votocek and Kohler, *Ber.*, **47**:1219 (1914).

<sup>8</sup>Thomas, *Compt. rend.* **181**:218 (1925).

<sup>9</sup>Lewis and Dufford, *J. Am. Chem. Soc.*, **45**:278 (1923).

<sup>10</sup>Dufford, Calvert and Nightingale, *J. Am. Chem. Soc.*, **45**:2058 (1923); **47**:95 (1925).

<sup>11</sup>Gilman and McCracken, *J. Am. Chem. Soc.*, **45**:2462 (1923).

<sup>12</sup>Gilman, Peterson and Schulze, *Rec. trav. chim.* **47**:19 (1928).

turnings was added at the start of the reaction. When all this magnesium had gone into solution, one equivalent of one of the following was added: ordinary magnesium, activated magnesium, ordinary or activated 2 per cent or 12.5 per cent copper-magnesium alloy. The yield of terephthalic acid in no case exceeded 1.25 per cent. Although the terephthalic acid was digested in alcohol, it always gave a Beilstein test<sup>13</sup>.

A Grignard reagent could not be formed with 1, 4-dibromobenzene in anisole or dimethylaniline as solvents, but on the addition of 25 cc. of ether a fair yield of 4-bromobenzoic acid was obtained, although terephthalic acid could not be detected.

*p*-Diiodobenzene. The Grignard reagent prepared in anhydrous ether from 12.2 g. (0.037 mole) of *p*-diiodobenzene and 1.8 g. (0.075 mole) of magnesium, after being treated with dry carbon dioxide, yielded 0.5 g. (33.8 per cent; 0.0124 mole) of terephthalic acid, 0.2 g. of *p*-diiodobenzene and 5 g. of an intractable oil.

*m*-Diiodobenzene. The Grignard reagent prepared from 16.5 g. (0.05 mole) of meta-diiodobenzene and 2.5 g. (0.1 mole) of magnesium yielded 4 g. (0.024 mole; 48.2 per cent) of isophthalic acid, identified as the dimethyl ester melting at 60-64°.

*Sym*-tribromobenzene. *Sym*-tribromobenzene could not be induced to react with magnesium, even on activating with iodine, bromine, ethyl bromide, bromobenzene, ordinary and activated 2 per cent and 12 per cent copper-magnesium alloys. Of the original halide 71.4 per cent was recovered.

*o*-Dibromobenzene. On adding an ether solution of 1.8 g. (0.05 mole) of *o*-dibromobenzene to 3 g. (0.12 mole) of magnesium, very little magnesium could be brought into solution. Toluene was added, after which the mixture was refluxed for 12 hours at 105°. The only product that could be isolated was *o*-bromophenol, which was identified by converting it into tribromophenol. A yield of one g. (31.7 per cent) was obtained, showing that a mono-Grignard reagent had been formed, but during the protracted refluxing it was converted into the phenate.

*m*-Dibromobenzene. A Grignard reagent was readily prepared from 11.8 g (0.05 mole) of *m*-dibromobenzene and 1.2 g. (0.05 mole) of magnesium. On adding 0.5 g. of 12.5 per cent copper magnesium alloy, when the reaction stopped, no further reaction could be induced to take place. Four grams (0.002 mole; 40 per cent) of *m*-bromo-benzoic acid was obtained.

## CONCLUSION

1. It has been shown that there is no analogy between Grignard reagent formation with polyhalogen compounds and Fry's electronic formula for benzene.

2. When dealing with polyhalogen aromatic compounds, there is no catalyst known that will induce the second halogen to react to form a di-Grignard reagent to a greater extent than can be accomplished without a catalyst. (See, however, Ref. 12.)

<sup>13</sup>Gilman, Beaber and Jones, *Rec. trav. chim.* **48**:597 (1929) reported on a continuation of this study.



## II. A STUDY OF ANTI-KNOCK COMPOUNDS

Polis<sup>1</sup> first prepared lead tetraphenyl by heating bromobenzene and a 4-6 per cent sodium-lead alloy in ethyl acetate. Pfeiffer and Truskier<sup>2</sup> later prepared it by the use of the Grignard reagent and lead chloride.

It was obtained in a 50.5 per cent yield following the directions of Grüttner<sup>3</sup>. Fifty-seventh-hundredths g. lead tetraphenyl was found to be soluble in 100 cc. nitrobenzene at 25°.

Several unsuccessful attempts were made to prepare lead tetra-alpha-naphthyl.

Solutions of lead tetraphenyl in nitrobenzene were made of such strengths that when added to a ten-gallon tank of gasoline they would produce the following concentrations, of lead teraphenyl: 1 part in 5,000, 1 part in 50,000, 1 part in 100,000, 1 part in 250,000 and 1 part in 500,000 and given to Dr. O. R. Sweeney, who had them tested. The preliminary report was that they were equally as good as lead tetra-ethyl as an antiknock.

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<sup>1</sup>Polis, *Ber.*, 20:717, 3332 (1887).

<sup>2</sup>Pfeiffer and Truskier, *Ber.* 37:1125 (1904).

<sup>3</sup>Grüttner, *Ber.*, 44:334 (1911); 49:1125 (1916); 51:1293, 1298 (1918).

# I. THE QUINHYDRONE ELECTRODE AND THE SOIL REACTION<sup>1</sup>

EMERSON R. COLLINS

*From the Department of Chemistry, Iowa State College*

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This section of the thesis<sup>2</sup> gives a short summary of the theory underlying the quinhydrone electrode, followed by a development of the fundamental equations, with particular emphasis on the temperature correction necessary when the quinhydrone electrode is used with the saturated KCl calomel half cell. Sufficient information is given so that it is a simple matter to calculate similar temperature corrections for other than the saturated KCl calomel half cell.

A convenient graph is presented for making temperature corrections rapidly when equation (A) is used.

$$\text{pH} = \frac{0.4526 - \pi}{.0591} \quad 25^{\circ}\text{C} \quad (\text{A})$$

If a large number of readings are to be made, it is a great saving of time to prepare a table of E. M. F. vs. pH values at 25 and then use the graph mentioned above for temperature variations. Corrections are added to the calculated pH if the temperature is below 25° and subtracted if the temperature is higher.

The following equation (B) may be used in place of the above equation (A) supplemented by the graph.

$$\text{pH} = \frac{0.4526 - 0.00009(t - 25) - \pi}{0.0591 + [0.002(t - 25)]} \quad (\text{B})$$

Where  $\pi$  = E.M.F. reading and  $t$  = centigrade temperature.

The experimental part of this paper deals with equilibrium conditions between the soil and water mixture. This includes a comparison of the Büllmann and Tovborg-Jensen's methods—mixing the soil and water by shaking for a few seconds, with such procedures as stirring by motor, by rotary shaker and by passing through a colloid mill. No definite equilibrium was found on the soils used.

Soils were passed through a colloid mill and then allowed to settle in a long tube with openings every foot. From the pH values of the samples withdrawn at different heights, evidence was presented to show that this treatment increased the differences between the pH of the soil

<sup>1</sup>An abstract of a thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup>Clark, N. A. and Collins, E. R. 1927. The quinhydrone electrode and the soil reaction. *Soil Sci.* 24:453-463.

and that of the supernatant liquid. This difference may be caused by a Donnan equilibrium set up in the soil, but regardless of the cause it emphasizes the question as to what pH value should be taken for the soil reaction.

## II. THE GRAVIMETRIC METHOD FOR THE DETERMINATION OF CARBONATES IN SOIL

This section of the thesis<sup>3</sup> treats of the determination of carbonates in soils, using 1 to 10 hydrochloric acid for decomposing the carbonates and adapts the mercury seal for stirring the soil-acid mixture in order to obtain better contact. Ascarite is used as the absorbent for the carbon dioxide liberated from the soil.

Hydrochloric acid was chosen because its action on the carbonates has been found to be as complete as the action of any acid can be without oxidizing the organic matter of the soil. This 1 to 10 acid was found to have altogether negligible action upon the organic matter with 5, 10, 20 gram charges of the average soil, when the determination was run at room temperature.

The necessity for shaking the soil with the acid has been noted previously and several methods have been presented such as the multiple shaking apparatus of MacIntire and Willis and Truog's method of bubbling air through the suspension. However none of these methods produces the vigorous action obtained by a stirrer, and for this reason the mercury seal as described by Brühl has been adopted for this purpose. The rapid agitation produced by the stirrer decreases the time required to free the solution and absorbing train of carbon dioxide and consequently reduces the time required for each determination.

The ascarite and dehydrite used for absorbing the carbon dioxide and moisture respectively are contained in a glass stopper U-tube, as the weight of the filled tube is relatively much less than the average Midvale absorption bulb and consequently can be weighed with a greater degree of accuracy.

The U-tube is wiped off with a cloth dampened with alcohol; then with a dry cloth; and finally with a chamois skin. It is placed in the balance case for a few minutes, to obtain equilibrium before weighing.

The purifying and absorbing train consists of the following:

1. Potassium hydroxide to remove  $\text{CO}_2$  from the air drawn through the train.
2. Concentrated sulfuric acid to dry the air before passing it through the next tube containing ascarite.
3. Ascarite to remove any trace of  $\text{CO}_2$  that might have passed through the potassium hydroxide solution.
4. Dropping funnel used for the addition of 1 to 10 hydrochloric acid.

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<sup>3</sup>Clark, N. A., and Collins, E. R. 1929. The gravimetric method for the determination of carbonates in soil. *Soil Sci.* 27:407-414.

5. Two hundred cc. round-bottom sample flask fitted with a three hole rubber stopper for the inlet and outlet tubes as well as an opening for the mercury seal.
6. Six-inch condenser to decrease the amount of volatile material that may be drawn over into the following purifying train.
7. Silver sulfate in 1.4 sulfuric acid for the removal of chlorides.
- 8, 9. Constant boiling sulfuric acid.
10. Dehydrite or phosphorus pentoxide absorbing agents are placed here before the ascarite, to be sure that the vapor pressure entering the CO<sub>2</sub> absorbing U-tube is the same as when it leaves the final drying agent of dehydrite or phosphorus pentoxide in the U-tube.
11. U-tube containing ascarite for the absorption of CO<sub>2</sub>, and dehydrite or phosphorus pentoxide to take up the moisture produced from the reaction between the CO<sub>2</sub> and the ascarite.
12. Calcium chloride guard tube.

The results from three representative soils contain widely differing amounts of carbonate are given. When the soil and carbonate, in the form of Iceland spar, were mixed, the CO<sub>2</sub> obtained from the soil, after the CO<sub>2</sub> from the Iceland spar is subtracted, is almost identically the same as it is when the soil only is used.

If the determination is run at 50°, there is a slight increase in the amount of CO<sub>2</sub> released when compared to the same soil run at room temperature. When CO<sub>2</sub> saturated water was aspirated in the presence of HCl at room temperature, no increase in CO<sub>2</sub> was obtained by raising the temperature to 50°C. Similarly the Iceland spar showed no increase in the weight of CO<sub>2</sub> in running at 50°C. This indicates that the increase, when soil is present, is caused by the action of the acid on the soil organic matter and not to any CO<sub>2</sub> dissolved in the water.

### III. APPLICATION OF THE QUINHYDRONE ELECTRODE TO INVESTIGATIONS ON BASE EXCHANGE IN SOILS

This part of the thesis is not published in complete form, but the principal parts are presented in a paper by Clark and Collins<sup>1</sup>.

The experimental data are presented from the results obtained on 21 distinct soil types of Iowa. Their description, location, origin, color, drainage and lime requirement are tabulated according to the information given in the soil survey reports of the respective counties.

The quinhydrone electrode was used for the determinations of pH, but was modified to avoid objections usually advanced when this electrode is used. Since a soil suspension shows a different pH in the supernatant liquid and in the settled soil, it was thought advisable to keep the soil in suspension with a mechanical stirrer. This is accomplished by placing a

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<sup>1</sup>Clark, N. A., and Collins, E. R. 1930. Equilibrium between soil and electrolytes, and its influence upon some lime requirement methods. *Soil Sci.* 29:417-427.



small stirrer between the Schollenberger calomel half cell and the platinum electrode. The Schollenberger electrode under the proper conditions with a good ground glass joint gives no noticeable diffusion when immersed in distilled water and held to the light. This precaution is necessary because even traces of KCl in the soil suspension give an appreciable change in the pH value measured.

The procedure followed used a one to five ratio of soil to water in every case. Sufficient quinhydrone was added to the solution and placed in contact with the platinum electrode while stirring. After a few seconds stirring, the electrode vessel was pushed up so that the Schollenberger electrode, which was at a higher level, was in contact with the solution and the E. M. F. reading taken at once.

Equilibrium conditions have been studied on the 21 Iowa soils under conditions of continuous stirring. This was made possible by the use of a multiple stirring device of 36 stirrers in which the solutions were protected from contact with the atmosphere. From the curves obtained with soil and water or with electrolytes such as calcium hydroxide, calcium or sodium chloride, it is difficult to draw any definite conclusions as to the optimum time of stirring necessary for equilibrium. Any definite time given for equilibrium conditions would not hold for all soils, regardless of whether water or electrolytes are used.

By the use of the stirring apparatus mentioned above, these 21 soils were titrated with  $\text{Ca}(\text{OH})_2$  by adding base of different normalities and stirring each sample for at least 90 hours. These results are plotted in the form of graphs showing the change in pH at frequent intervals after the base was added. Similar curves are shown for the titration of the soil with  $\text{Ca}(\text{OH})_2$  in the presence of 0.4 N  $\text{CaCl}_2$  and with NaOH in the presence of 0.4 N NaCl.

Considering the lime requirement of a soil as the amount of base required to bring the soil to pH 7.0, the three titration methods given above were compared as to their relative value as a lime requirement method. The three methods gave results approximately in the same order but varying in intensity. The titration with  $\text{Ca}(\text{OH})_2$  in a water suspension gives the lowest values for lime requirement and NaOH in 0.4 N NaCl second. In all cases the value obtained for  $\text{Ca}(\text{OH})_2$  in 0.4 N  $\text{CaCl}_2$  was higher than either of the other two.

It is important to observe that the lime requirement increased very rapidly as the time of stirring was lengthened, and the values obtained shortly after the addition of the base are not equilibrium conditions.

With one exception, the lime requirement as determined by titration with  $\text{Ca}(\text{OH})_2$  in 0.4 N  $\text{CaCl}_2$ , measured on mixing, was two to three times as great as when determined by titration with  $\text{Ca}(\text{OH})_2$  alone; measured at 90 hours, the requirement was 2.5 to 5.5 times as great. These figures are in the order of Christensen and Jensen's measurements, in which they found that approximately three times as much lime was needed in the field as was shown by the simple titration method with  $\text{Ca}(\text{OH})_2$ .

A modified procedure is given for the Williams method of determining base exchange in order that it may be applied to soil containing carbonates. The method consists of replacing the cations on the complex with 0.5 N acetic acid and then analyzing the solution for the ions replaced. This method was modified for carbonate soils by determining the  $\text{CO}_2$  liber-

ated from the soil with 0.5 N acetic acid using the apparatus of Clark and Collins<sup>5</sup>.

Making use of the suggestion of Bollen and Neidig and expressing the

results in equivalent parts per million,  $\text{Eq. P.P.M.} = \text{P.P.M.} \times \frac{1}{\text{eq. wt.}}$ ,

we can calculate the total base exchange of calcium and magnesium by subtracting the equivalent parts per million of  $\text{CO}_2$  from the total equivalent parts per million of calcium and magnesium obtained by the modified Williams method. The results so obtained are recorded for the 21 soils described.

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<sup>5</sup>Clark, N. A., and Collins, E. R. 1929. The gravimetric method for the determination of carbonates in soil. *Soil Sci.* 27:407-414.



# INSECTICIDAL ACTION IN THE NITROGEN HETEROCYCLICS<sup>1</sup>

LYMAN C. CRAIG

*From the Department of Chemistry, Iowa State College*

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## INTRODUCTION

Studies on nicotine<sup>2</sup>, one of the few organic compounds having high toxicity to insects, have failed to reveal a specific atomic linkage responsible for the high toxicity. Accordingly chemists as well as entomologists have come to believe that the high toxicity is caused by the make up of the molecule as a whole rather than to any single linkage or grouping.

The investigations reported in this work were undertaken on the assumption that if a series of pyrrolidine derivatives were prepared and their dissociation constants measured as well as their toxicity to insects, a gradient correlation would be found between the two sets of data. The dissociation constants would be expected to correlate with the former relationship reported by Hixon and Johns<sup>3</sup> between the organic amines and acids and would be a distinct contribution in the field of chemistry even if they failed to throw light on the cause of the high toxicity in nicotine.

## EXPERIMENTAL

A number of pyrrole compounds were prepared by pyrogenic synthesis<sup>4</sup> from substituted amino salts of mucic acid. Under the same conditions of synthesis, the comparative yields were the following:

Pyrrole .....	32 per cent
N-phenylpyrrolidine .....	25 per cent
N-methylpyrrole .....	22 per cent
N-n-butylpyrrole .....	14 per cent

These compounds were used as intermediates in the synthesis of pyrrolidine compounds.

A suitable method of synthesis for  $\alpha$ -substituted pyrrolines has been worked out by slightly modifying the rearrangement of  $\gamma$ -chloropropylketimines to pyrrolines first reported by Cloke<sup>5</sup>. This modification consists in heating the reaction product of  $\text{RMgX}$  with  $\gamma$ -chlorobutyronitriles, thereby

<sup>1</sup>An abstract of a thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup>(a) Harlan, Studies on Nicotine (Ph. D. thesis), Iowa State College Library; (b) La Forge, J. Am. Chem. Soc., **50**:2471 (1928); (c) Richardson and Smith, United States Department of Agriculture, Bull. 1160 (1923); (d) Tattersfield and Gimmingham, Ann. Appl. Biol., **14**:217 (1927); (e) Richardson and Shepard, J. Agr. Res., **40**:1007 (1930).

<sup>3</sup>Hixon and Johns, J. Am. Chem. Soc., **49**:1786 (1927).

<sup>4</sup>Schwanert, Ann., **116**:287 (1860); Bell and Lapper, Ber., **10**:1861 (1877); Feist, Ibid., **35**:1655 (1902).

<sup>5</sup>Cloke, J. Am. Chem. Soc., **51**:1174 (1929).



splitting out  $\text{MgXCl}$  with closure of the ring to form  $\alpha$ -substituted pyrrolines.  $\alpha$ -phenylpyrroline,  $\alpha$ -ethylpyrroline and  $\alpha$ -benzylpyrroline were synthesized by this method.

Pyrrolidine, N-methylpyrrolidine, N-n-butylpyrrolidine and N-cyclohexylpyrrolidine were prepared by catalytic reduction of the corresponding pyrrole derivatives with Adams and Shriner platinum oxide platinum black catalyst. The solvent employed was alcohol acidified with hydrochloric acid. This solvent offered an improvement over the solvents previously used in this type of reduction<sup>6</sup>. A synthesis of the above mentioned N-substituted pyrrolidines was attempted by treatment of pyrrolidine with  $\text{RX}$ , but the yields were very low. However, the compound, N-benzylpyrrolidine, first reported by Schlinck<sup>7</sup>, was synthesized by this method.

N-phenylpyrrolidine and N-para-tolylpyrrolidine were prepared by condensation of aniline and para-toluidine with tetramethylene chloride.

$\alpha$ -ethylpyrrolidine and  $\alpha$ -phenylpyrrolidine were prepared by reduction of the corresponding  $\alpha$ -substituted pyrroline, the first catalytically as the pyrrole derivatives above were reduced and the latter chemically by means of tin and hydrochloric acid according to the directions of Gabriel and Coleman<sup>8</sup>. It was found impossible to isolate  $\alpha$ -phenyl-N-methylpyrrolidine by methylation of  $\alpha$ -phenylpyrrolidine as reported by La Forge<sup>2</sup>.

$\alpha$ -picolylamine and  $\beta$ -picolylamine were prepared by reduction of the corresponding pyridylmethanaldoximes by use of zinc and acetic acid in ethyl alcohol as a solvent.

The dissociation constants of some of these bases, which were deter-

TABLE 1. *Dissociation constants of a few nitrogen heterocyclics*

Compound	E.M.F. against sat. calomel	Purity mol. wt. theory found		Acid added	Dissocia- tion constant
Pyrrolidine	.9009	71.1	71.3	$\frac{1}{2}$ equiv.	$1.3 \times 10$
N-cyclohexylpyrrolidine	.8787	153.19	153	$\frac{1}{2}$ equiv.	$5.0 \times 10$
N-benzylpyrrolidine	.8070	161.12	161.7	$\frac{1}{2}$ equiv.	$3.2 \times 10$
N-methylpyrrolidine	.8474	85.1	85.4	$\frac{1}{2}$ equiv.	$1.5 \times 10$
N-n-butylpyrrolidine	.8581	127.1	127.1	$\frac{1}{2}$ equiv.	$2.3 \times 10$
N-phenylpyrrolidine	.4490	-----	-----	1 equiv.	$2 \times 10$
N-p-tolylpyrrolidine	.4772	-----	-----	1 equiv.	$5 \times 10$
$\alpha$ -ethylpyrrolidine	.8630	99.1	99	$\frac{1}{2}$ equiv.	$2.7 \times 10$
$\alpha$ -phenylpyrrolidine	.8143	147.1	147.3	$\frac{1}{2}$ equiv.	$4 \times 10$
$\beta$ -pyridyl- $\alpha$ -N-methyl- pyrrolidine	.7157	162.2	162	$\frac{1}{2}$ equiv.	$9 \times 10$
$\alpha$ -picolylamine	.7220	108.1	108	$\frac{1}{2}$ equiv.	$1.1 \times 10$
$\beta$ -picolylamine	.7165	108.1	108	$\frac{1}{2}$ equiv.	$1 \times 10$
$\alpha$ -ethylpyrroline	.6850	97.1	96.8	$\frac{1}{2}$ equiv.	$3.7 \times 10$
N-phenylpiperidine	.4762	-----	-----	1 equiv.	$1 \times 10$

<sup>6</sup>Willstatter and Hatt, Ber., 45:1477 (1912); Hess, Ibid., 46:3113 (1913); Wibaut, Rec. trav. chim., 44:1101 (1925); 49:237 (1930); Anderson and McElvain, J. Am. Chem. Soc., 51:887 (1929).

<sup>7</sup>Schlinck, Ber., 32:952 (1899).

<sup>8</sup>Gabriel and Coleman, Ber., 41:517 (1908).

mined either by the method of Carothers, Bickford and Hurwitz<sup>9</sup> or a slight modification of it, are presented in table 1.

The data supports the theory of electron sharing ability of organic radicals as set forth in the first paper of the series by Hixon and Johns<sup>3</sup>. If the above data are plotted in the same manner as were the data in the first paper of their series, a curve is obtained for the N-substituted pyrrolidines whose slope is identical with the slope of the primary amine curve. By plotting in like manner a series of  $R(CH_2NH_2)$  bases a value was placed on the electron sharing ability of  $\alpha$ - and  $\beta$ -pyridyl radicals. They were found to be extremely negative. The slope of the  $\alpha$ -substituted pyrrolidine curve was identical with that of the  $R(CH_2NH_2)$  curve.

The three bases,  $\alpha$ -picolylamine,  $\beta$ -picolylamine and nicotine were titrated electrometrically. The curves so obtained showed that they were monoacidic bases, the pyridine nitrogen was presumably without basic properties.

#### MEASUREMENT OF INSECTICIDAL ACTION

The methods for the measurement of relative toxicity employed in the past have not been sensitive enough for the detection of small differences in relative toxicity. A more sensitive method was desired. Such a method was developed whereby the convenient test insect, *Tribolium confusum* Duval, was exposed to a certain concentration of the compound in gaseous form for a recorded time. More uniform dosage could be obtained in this way than by previously used methods<sup>2d, 10</sup>. Relative toxicities were based on relative concentrations necessary to give a fifty per cent kill when the time of exposure was two hours and thirty-eight minutes. The fifty per cent kill point was determined by the statistical method of Snedecor and Wallace<sup>11</sup>.

The data are presented in table 2.

TABLE 2. *Results*

Compound	Conc. in g. per liter giving 50 per cent kill in 2 hr. 38 min.	Dissociation constant
Pyridine	.0346	$10^{-10}$
N-methylpyrrolidine	.0095	$1.5 \times 10^{-4}$
N-n-butylpyrrolidine	.0017	$2.3 \times 10^{-4}$
Pyrrolidine	.00109	$1.3 \times 10^{-3}$
N-phenylpyrrolidine	greater than .000408	$2 \times 10^{-10}$
$\alpha$ -phenylpyrrolidine	.000245*	$4 \times 10^{-8}$
Nicotine	.0000306	$9 \times 10^{-7}$

\*This result is not on the basis of fifty per cent kill, but is approximately correct. At maximum concentration obtainable a 25 per cent kill was effected in the time allowed. For all the compounds tested the change in the concentration from 25 per cent to 50 per cent was very slight.

<sup>9</sup>Carothers, Bickford and Hurwitz, J. Am. Chem. Soc., 49:2908 (1927).

<sup>10</sup>Shepard and Richardson, Unpublished MS.

<sup>11</sup>Correlation and Machine Calculation, Official Publication, Iowa State College, Vol. 23, No. 35, 47 pp. (1925).

It has been suggested that there might be a correlation between the dissociation constants and toxicity<sup>2a</sup>. This study shows that if such is the case, there is a maxima as we go from the stronger bases to the weaker. It seems more probable when the chemical properties of the series of compounds is surveyed, that there is a particular range of negativity of radicals where amines are unstable or are more reactive and that toxicity is related only to basicity as basicity is related to chemical reactivity. Far reaching conclusions cannot be drawn until the data are expanded.

# PROBLEMS RELATED TO THE COMMERCIAL PRODUCTION OF LEVULOSE<sup>1</sup>

JACK W. EICHINGER, JR.

*From the Department of Chemistry, Iowa State College*

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## INTRODUCTION

Levulose or d-fructose ( $C_6H_{12}O_6$ ), although possessing properties which should make it more valuable than the ordinary sugars of commerce, has not yet been produced on a commercial scale. An improved process incorporating several economic advantages has been developed at Iowa State College. Details of the process have been given by McGlumphy<sup>2</sup>, and the work of adapting it to semi-commercial production is under way. During the course of this work, many problems have arisen. Some of these problems were quickly solved, but others have required an extended investigation. Of the latter, the matter of the conversion of the substances of the inulin group, together with the small amount of sucrose contained in artichoke juices, was one of the first to come to our attention.

The purpose of this paper is to present experimental data designed to show the relations between the factors controlling the conversion process and thus make it possible to convert large batches of artichoke juice conveniently and economically.

## HISTORICAL

Jackson, Silsbee and Proffitt<sup>3</sup> have made a study of the conversion of artichoke juices. They measured a number of velocity constants and observed that the velocities vary considerably with the composition and concentration of the juice. Jackson's interpretation was that, for a more concentrated juice, a greater amount of acid was rendered ineffective by the inorganic impurities and the velocity constants for given strengths of acid were therefore lower.

The above investigators performed several experiments to determine the destructive effect of sulfuric acid at temperatures of 70°C. and 100°C. on levulose, and concluded that appreciable quantities of the levulose were destroyed.

## EXPERIMENTAL

Jackson, Silsbee and Proffitt<sup>3</sup> have shown that the resultant of the various reactions occurring during the conversion process follows substantially the course of a unimolecular reaction. Therefore, it is possible to measure the velocity constants of the rates of conversion of artichoke juices for

<sup>1</sup>An abstract of a thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup>McGlumphy, Problems related to the commercial production of levulose. Thesis, Iowa State College Library, Ames, Iowa (1930).

<sup>3</sup>Jackson, Silsbee and Proffitt, U. S. Bur. Standards Sci. Papers, 519:614 (1926).

different conditions of acidity and temperature and for different concentrations and compositions of juice. This furnishes the most satisfactory method of attack for this problem.

In order to determine the velocity constant for the rate of conversion, a quantity of juice was heated and maintained at the desired temperature. The required quantity of acid was added and portions withdrawn from time to time for polarization. Clarification was accomplished by means of a solution of normal lead acetate, as recommended by Jackson, Silsbee and Proffitt.

A number of titrations were made on raw and converted juices in order to test Jackson's theory regarding the neutralization of some of the acid by inorganic constituents of the juices, and the results are presented in table 1.

TABLE 1.

Percentage of total solids	7.2	14.4	18.0	26.1
Raw juice	0.0143	0.0238	0.0306	0.0445
Acid added	0.0950	0.0950	0.0950	0.0950
Total	0.1093	0.1188	0.1256	0.1395
Actual titration	0.1020	0.1088	0.1212	0.1292
Difference	0.0073	0.0100	0.0044	0.0103

From these data, we conclude that the actual titratable acidity of a converted juice is practically equal to the initial acidity of the raw juice plus that of the acid added.

TABLE 2. Velocity constants for the rate of conversion of artichoke juices at 80°C and different conditions of concentration and acidity.

Juice percentage of total solids	Apparent acidity	Velocity Constant	pH (quinhydrone electrode)
7.1	0.0782 N. HCl	0.0986	1.80
7.2	0.0950 " "	0.1680	1.68
14.4	0.0475 " "	0.0009	4.10
"	0.0950 " "	0.0136	2.80
"	0.1425 " "	0.0690	2.09
"	0.1900 " "	0.2310	1.55
18.0	0.0950 " "	0.0053	3.19
21.8	0.2160 " "	0.0984	1.86
22.3	0.1750 " "	0.0262	2.49
23.3	0.2670 " "	0.1650	1.51
26.1	0.0950 " "	0.0015	3.83
7.05	0.1140 N. H <sub>2</sub> SO <sub>4</sub>	0.0766	1.71
14.4	0.0573 " "	0.0013	3.92
"	0.1147 " "	0.0109	2.77
"	0.1720 " "	0.0513	1.95
"	0.2294 " "	0.1270	1.61
26.6	0.3710 " "	0.1180	1.50
30.5	0.3940 " "	0.2170	1.23
30.6	0.3150 " "	0.0973	1.45
30.7	0.2364 " "	0.0433	1.94
30.8	0.1560 " "	0.0107	2.77



By plotting the pH values against the velocity constants, two curves are obtained, one for each acid. It is evident from the curves that the velocity constant is a function of pH only for either acid. The fact that the two acids form separate curves is presumably to be attributed to the relative influences of the negative ions.

In order to be able to produce any desired value of hydrogen ion concentration in a juice of any given concentration, a number of experiments were performed. A series of different concentrations of juices were made up, various proportions of acid added, and the pH values measured by means of the quinhydrone electrode. The results are presented in table 3.

TABLE 3.

Juice		H <sub>2</sub> SO <sub>4</sub>			HCl		
Pctg.T.S.		A	B	C	A	B	C
4.8	Normality	0.1016	0.1916	0.0479	0.0768	0.1536	0.0384
	pH	1.62	1.31	2.36	1.60	1.16	2.54
8.65	Normality	0.0958	0.1916	0.1437			
	pH	2.07	1.42	1.61			
12.8	Normality	0.0977	0.2894	0.1916	0.1536	0.2304	0.0768
	pH	2.62	1.29	1.61	1.56	1.13	3.09
16.7	Normality	0.1916	0.2875	0.2396			
	pH	1.87	1.40	1.57			
19.9	Normality	0.1916	0.3833	0.2875	0.2304	0.3072	0.1536
	pH	2.16	1.27	1.54	1.49	1.07	2.48
23.0	Normality	0.2875	0.3833	0.3354			
	pH	1.71	1.32	1.46			
25.5	Normality	0.3833	0.2875	0.4791	0.3840	0.2304	0.3072
	pH	1.45	1.90	1.23	1.00	2.08	1.35
34.2	Normality	0.4791	0.3833	0.4312			
	pH	1.43	1.79	1.59			

When the pH values were plotted against the normalities, a series of curves were obtained for each acid. By interpolation and extrapolation, it is possible to find the normality of either acid required to produce any desired pH value in a juice of any given concentration.

A number of experiments were performed to determine the destructive effect of conditions of high acidity and temperature upon levulose. Solutions of levulose were acidified and maintained at 80 and 90°C. From time to time, portions were removed and polarized. It was found that at temperatures up to 80°C. and pH values down to 1.12, the amount of levulose destroyed in 60 minutes was insignificant.

#### SUMMARY

The factors controlling the conversion reaction have been determined.

The decomposition of levulose under conditions of high acidity and temperature has been studied, and the limiting conditions have been determined.

Conversion experiments under varying conditions of concentration and acidity have been run, and the data are presented in a table.

Normality-pH data for different concentrations of juice treated with varying quantities of sulfuric and hydrochloric acids have been determined.



# THE EFFECT OF CHROMIUM ON WHITE CAST IRON<sup>1</sup>

CLARENCE DALE FOULKE

*From the Department of Chemistry, Iowa State College*

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The present study was undertaken with a two-fold object in view: First, to make a quantitative study of the effect of chromium on graphitization rates of white cast iron; and, second, to determine the graphitization rates of alloys containing constant amounts of chromium with varying amounts of silicon.

## EXPERIMENTAL

As a preliminary study of the effect of chromium on the stability of cementite, three series of alloys of varying percentages of chromium but approximately constant amounts of other elements were prepared. Table 1 gives the chemical composition of these alloys.

TABLE 1. *Chemical composition of alloys*

Alloy no.	Percentage carbon	Percentage chromium	Remarks
A-1	2.20	0.339	alloys cast vertically in magnesia moulds
A-2	2.23	0.656	
A-3	2.18	0.862	
A-4	2.24	1.260	
A-5	2.20	1.477	
B-1	1.90	0.088	alloys cast vertically in magnesia moulds
B-2	1.88	0.181	
B-3	1.85	0.287	
C-1	2.26	0.033	alloys cast vertically in dry sand moulds
C-2	2.20	0.062	
C-3	2.23	0.102	

A sample of each alloy was placed in a graphite crucible, packed with powdered graphite, heated to and annealed at a temperature of 1700° F. for 70 hours. After cooling to 1100° F. during an interval of 13 hours, the alloys were removed from the annealing furnace and allowed to cool to room temperature.

A thorough microscopic examination was made of the heat treated alloys and photographs were taken. An alloy of approximately the same chemical composition as series A, but containing no chromium, was completely graphitized by this heat treatment. The alloy containing 0.033 per cent chromium (C-1) retained no free cementite, while 0.062 per cent

<sup>1</sup>An abstract of a thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

chromium (alloy C-2) retained traces of cementite even after this prolonged heat treatment.

From the results obtained by the preliminary anneal it was concluded that chromium, even in small amounts, has a very pronounced stabilizing effect on the cementite of white cast iron at 1700° F. Since silicon has a powerful graphitizing effect on cementite at this temperature, it was decided to prepare several series of chromium bearing alloys and study the effects of constant chromium content and increasing silicon content on rates of graphitization.

About 25 pounds of stock alloy were prepared for each series by melting white cast iron test bars and adding the calculated amount of ferrochrome. The several alloys of each series were then prepared from the stock alloy by remelting two to three pound portions, adding definite amounts of ferro-silicon and casting in air dried sand moulds. A weighed quantity of 20 mesh graphite was added to each melt to compensate for oxidation losses. In order to have some basis with which to compare the relative graphitization rates of the different alloys, one bar (alloy 0) containing no chromium, was prepared under the same conditions as obtained in the preparation of the other alloys. The alloys were analyzed for carbon, chromium, silicon, manganese, sulphur and phosphorus, using methods recommended by the American Society for Testing Materials.

Samples of each alloy and of each series were placed in iron pipes (2 x 8 inches), packed with powdered graphite and heated to a temperature of 1700° F. The samples of higher silicon content were removed from the furnace at five hour intervals, while those of low silicon content were removed at 10 hour intervals. Each sample was examined microscopically and the times required for the complete graphitization of the cementite determined in each case. In order to effect the graphitization of the eutectoid cementite several samples of each alloy were replaced in the annealing furnace and held at 1700° F. for the times necessary for the graphitization of the free cementite. The furnace and samples were then cooled to a temperature of 1300° F.; this required a period of eight hours. After being at 1300° F. for definite lengths of time, samples were removed and examined microscopically. The complete absence of pearlite was taken as the criterion of complete graphitization.

Table 2 shows the times required for the graphitization of the free cementite and the eutectoid cementite. The times stated are those during which the alloys were held at 1700° F. and 1300° F., respectively, and do not include the eight hours required in cooling from 1700° F. to 1300° F.

One series of alloys, the M series, containing 0.43 per cent chromium and silicon varying from 0.83 per cent to 1.91 per cent, retained considerable free cementite after 200 hours of annealing at 1700° F. The complete graphitization of this series of alloys was not attempted. Each of the alloys D-2, containing 0.026 per cent chromium and 0.88 per cent silicon; F-8, containing 0.084 per cent chromium and 1.13 per cent silicon; E-9, containing 0.112 per cent chromium and 1.17 per cent silicon; and N-5, containing 0.188 per cent chromium and 1.57 per cent silicon, require approximately the same number of hours for complete graphitization as does the reference alloy, C-1. If we assume that alloy C-1 is fairly representative of white cast iron produced in commercial practice, we can conclude that for alloys containing 0.026 per cent chromium the presence of at least 0.88 per cent

TABLE 2. *Times of complete graphitization of alloys*

Alloy	Weight percentage		Times for complete graphitization	
	Cr	Si	Time at 1700° F.	Time at 1300° F.
C-1	0.000	0.82	25 to 30 hours	35 to 40 hours
D-1	0.025	0.81	30 to 40 hours	50 to 60 hours
D-2	0.026	0.88	20 to 30	30 to 40
D-3	0.026	0.90	20 to 30	30 to 40
D-4	0.026	0.93	20 to 30	30 to 40
D-5	0.026	1.05	10 to 20	20 to 30
D-6	0.026	1.06	10 to 20	20 to 30
D-7	0.026	1.09	10 to 15	10 to 20
D-8	0.026	1.16	5 to 10	10 to 20
D-9	0.025	1.20	5 to 10	10 to 20
F-1	0.086	0.84	60 to 70 hours	110 to 120 hours
F-2	0.086	0.93	50 to 60	80 to 90
F-3	0.086	0.94	50 to 60	70 to 80
F-4	0.085	0.96	50 to 60	70 to 80
F-5	0.084	1.01	40 to 50	50 to 60
F-6	0.084	1.08	40 to 50	50 to 60
F-7	0.084	1.07	30 to 40	50 to 60
F-8	0.084	1.13	20 to 30	30 to 40
E-1	0.111	0.86	70 to 80 hours	140 to 150 hours
E-2	0.113	0.91	70 to 80	140 to 150
E-3	0.113	0.96	60 to 70	130 to 140
E-4	0.112	0.99	60 to 70	120 to 130
E-5	0.114	1.04	50 to 60	90 to 100
E-6	0.108	1.07	40 to 50	90 to 100
E-7	0.101	1.08	40 to 50	90 to 100
E-8	0.111	1.14	20 to 30	80 to 90
E-9	0.112	1.17	10 to 20	50 to 60
N-1	0.189	0.84	130 to 150 hours	More than 160 hr.
N-2	0.186	1.01	70 to 90	More than 150 hr.
N-3	0.186	1.26	40 to 50	More than 140 hr.
N-4	0.197	1.44	20 to 30	90 to 100 hours
N-5	0.188	1.57	15 to 20	70 to 80
N-6	0.185	1.89	5 to 10	40 to 50
N-7	0.184	2.35	5 to 10	5 to 10

silicon is advisable as regards graphitization rates. This comparison may be carried through the F, E and N series of alloys.

Photographs were taken of representative alloys of the several series showing the structures as cast and the changes in micro-structure produced in progressive annealing of these alloys. Photographs of alloys D-9, N-6 and N-7 show the only alloys of the entire group in which primary graphitization was produced while cooling in the mould.



## CONCLUSIONS

Chromium, when present in white cast iron, exhibits a very marked stabilizing effect on combined carbon. That the combined carbon of the eutectoid as well as that of the free cementite is stabilized, is shown by the greatly extended times required for graphitization of chromium bearing alloys, both at 1300° F. and 1700° F.

Silicon, by its powerful graphitizing tendency, is an effective agent for correcting the inhibiting effect of chromium. However, in order to prevent the separation of primary graphite during solidification, an upper limit of silicon for each chromium content must not be exceeded. For 0.025 per cent chromium alloys this upper limit is between 1.15 and 1.20 per cent silicon, while for 0.18 per cent chromium alloys this limit is about 1.80 per cent silicon.

When large amounts of scrap are used in the manufacture of pig iron, it is practically impossible to hold the chromium content much below 0.05 per cent. By adding the proper amounts of silicon such pig iron can be annealed satisfactorily, resulting in a marked saving to the manufacturer of malleable iron.

No attempt has been made to correlate physical properties with chemical constitution. It is quite possible that the alloys of higher silicon content, that is, those requiring relatively short annealing periods, would show inferior physical properties.

## SUMMARY

1. A study of the quantitative effects of chromium on cementite stability has been made.
2. Rates of first and second graphitization stages have been determined for several alloy series containing constant amounts of chromium and varying amounts of silicon.

# CHEMICAL STERILIZATION WITH ALKALIES<sup>1</sup>

JAMES ROGER HALL

*From the Department of Chemistry, Iowa State College*

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It has been generally believed that the germicidal efficiency of an alkali was a direct function of the concentration of hydroxyl ions. Some doubt was thrown on this conception by the work of Levine, Buchanan, and Toulouse<sup>2</sup>, who maintained that the concentration of undissociated alkali molecules constituted an important factor. These investigators reported that the addition of sodium salts markedly increased the germicidal efficiency of sodium hydroxide, although the hydroxyl ion concentration as determined electrometrically was not measurably altered. The effect of temperature and concentration of alkali on the germicidal efficiency has been investigated by Levine, Buchanan, and Lease<sup>3</sup>. An excellent review of the literature on disinfection with alkalies is given by Meyers<sup>4</sup>.

The purpose of these experiments was to investigate the relative germicidal efficiencies of the caustic alkalies and to determine the relative influence of the addition of the various sodium salts on the germicidal efficiency of sodium hydroxide.

The technique used was a slight modification of that developed by Levine, Buchanan, and Lease<sup>3</sup> in their original work. A new flask was devised to take the place of the three-neck Woulff flask used previously, and a mixed indicator described by Lizius<sup>5</sup> was employed in place of the methyl orange indicator used in the original technique.

The organism used was the same as that employed in the original work. It was described as a spore former of the *B. subtilis* group and very resistant to alkali. Two different dried spore preparations were used. In the first experiments, an older spore preparation was used which had lost some of its vitality. For this reason, the tests were carried out at 40°C. In the meantime, a new dried spore mixture was prepared and it was found that these spores were more resistant to alkali, so the temperature was raised to 50°C. With either spore preparation the results were made comparable by including in each experiment a control test solution of sodium hydroxide.

Previous investigators<sup>6</sup> reported that lithium, sodium, and potassium hydroxides have very similar germicidal powers. In this investigation, the hydroxides of lithium, sodium, potassium, and rubidium were tested and it was found that at the same normality, the germicidal efficiencies were the same. An equal mixture of sodium hydroxide and potassium hydroxide

<sup>1</sup>An abstract of a thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup>Levine, Buchanan and Toulouse, *Iowa State College Jour. Sci.*, 2:19-29 (1927).

<sup>3</sup>Levine, Buchanan, and Lease, *Iowa State College Jour. Sci.*, 1:379-394 (1927).

<sup>4</sup>Myers, *J. Bact.*, 15:341-356 (1928).

<sup>5</sup>Lizius, *Analyst*, 46:355-356 (1921).

<sup>6</sup>Paul and Krönig, *Z. physik Chem.*, 21:414-450 (1896).

was tested against sodium hydroxide at the same normality, and it was found that the germicidal properties were practically identical.

Considering the killing time of a solution to be the time in which 99.9 per cent of the test organisms introduced were killed, it was found that in four experiments with lithium hydroxide at 40°C., an average killing time of 47.2 minutes was observed, whereas the sodium hydroxide controls showed an average killing time of 47.4 minutes.

An average of the killing times in three experiments with potassium hydroxide at 40°C., gave 54.9 minutes compared to 55 minutes for the sodium hydroxide controls.

Three experiments with the potassium hydroxide-sodium hydroxide mixture at 40°C. showed an average killing time of 55.5 minutes compared to 55 minutes for the sodium hydroxide controls.

Rubidium hydroxide in four experiments at 50°C. gave an average killing time of 34 minutes compared to 33.9 minutes for sodium hydroxide.

In each case, the killing time for the sodium hydroxide and the test solutions were practically the same.

In determining the effect of the addition of several sodium salts on the germicidal powers of sodium hydroxide, two different amounts of each salt were employed. For the first set of observations in each case, an amount of the salt was used which contained one-half as much sodium as was contained in the 100 cc. of 0.5 N sodium hydroxide used in the experiment. In the second set of tests, twice as much of the salts were added. Suitable controls of sodium hydroxide were included in each experiment. The killing times found are given in the following table (table 1).

TABLE 1. *Effect of the addition of sodium salts on the germicidal efficiency of sodium hydroxide*

Concentration of added salt*	0.0	0.025	0.050
Salt employed	Killing time in minutes		
NaCl	35.3	31.0	24.8
	35.5	31.3	25.8
NaNO <sub>3</sub>	35.3	30.8	25.0
	36.0	29.7	24.8
Na <sub>2</sub> CO <sub>3</sub>	35.5	29.5	25.5
	35.8	29.5	25.5
Na <sub>2</sub> SO <sub>4</sub>	34.7	29.0	25.3
	34.6	29.5	25.8
Na <sub>2</sub> SiO <sub>3</sub>	35.9	25.8	21.9
	35.1	26.0	21.1
Na <sub>3</sub> PO <sub>4</sub>	35.2	29.2	25.3
	35.4	29.8	24.7

\*Expressed in mols of sodium per 100 cc. of 0.5 N NaOH.

From an inspection of table 1, it will be seen that the salts gave nearly the same reduction in killing time, with the exception of sodium silicate.

The higher alkalinity which sodium silicate is known to possess over the others salts tested would account for its greater germicidal efficiency.

It is suggested that the principal factors in disinfection with alkalies are the concentrations of hydroxyl ions and undissociated sodium hydroxide molecules.





# PROBLEMS RELATED TO THE COMMERCIAL PRODUCTION OF LEVULOSE<sup>1</sup>

JAMES H. MCGLUMPHY

*From the Department of Chemistry, Iowa State College*

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## INTRODUCTION

Levulose possesses distinctive properties which earn for it a place among the commercial sweetening agents. It is the sweetest of all the sugars<sup>2</sup>, and one of the most soluble<sup>3</sup>. It is somewhat more hygroscopic than sucrose or dextrose<sup>4</sup>. Levulose possesses unique physiological properties, being resorbed directly, assimilated to a larger extent and oxidized more quickly and in larger amounts per time interval than sucrose<sup>5</sup>.

The addition of levulose to the list of already available sugars should give any desired combination of sweetness, texture and solubility for the production of food products. In jellies, marmalades, and preserved fruits, levulose would prevent the crystallization of sucrose<sup>6</sup>. The presence of levulose in ice cream, ices, etc., might prevent the crystallization of lactose, which gives considerable trouble in the ice cream industry. A greater degree of delicacy could be obtained in many confectionary products by using levulose alone or in combination with glucose or sucrose. It should be ideal for the manufacture of candies because of its ability to prevent crystallization of other sugars in its presence. The use of levulose would give a uniform and permanent flavor to carbonated beverages by preventing the loss of sweetness in the bottled product caused by inversion. Artificial honey could be prepared by using levulose in combination with dextrose. The use of dextrose could be greatly increased by mixing it with crystalline levulose and thus raising its sweetening power. Many additional advantages may be discovered for levulose when it is produced in sufficient quantities to allow experimentation on its culinary and confectionary applications.

Perhaps the most important uses for levulose are medicinal uses. It is an excellent food for the infant<sup>7</sup>. It is valuable as a food for the consumptive where the production of CO<sub>2</sub> in abundance is important<sup>4</sup>. It is effective in the prevention of hyperacidity of the gastric juice<sup>6</sup>.

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<sup>1</sup>An abstract of a thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup>Paul, *Chem. Zeit.*, **45**:705 (1921), reports that if the sweetness of sucrose is 100, levulose is 103. Sale and Skinner, *J. Ind. Eng. Chem.*, **14**:522 (1922), state that, on the same basis, levulose is 150. According to Beister, Wood and Wahlin, *Amer. J. Physiol.*, **73**:387 (1925) levulose is 173.

<sup>3</sup>Jackson, Silsbee and Proffitt, *Bur. Standards Sci. Papers*, **519**:614 (1926), report 375 g. levulose soluble in 100 g. water at 20° C.

<sup>4</sup>Browne, *J. Ind. Eng. Chem.*, **14**:712 (1922).

<sup>5</sup>Daniel, *Chem. Zeit.*, **45**:4 (1921).

<sup>6</sup>Stein, *ibid.*, **32**:426 (1908).

Many diabetic patients tolerate levulose to a remarkable extent<sup>7</sup>. Even with insulin treatment there is need for carbohydrate foods. Levulose not only supplies this need, in part, but satisfies the desire for sweets without the use of substitutes which have little or no food value, or which may even be harmful.

There seems to be a direct correlation between the increase in the per capita consumption of sugar (cane and beet), and the increase in the number of deaths, per one hundred thousand population, from diabetes<sup>8</sup>. Sugar (sucrose) has never been shown to cause diabetes. However, if the condition is present, the fact is well known that it is greatly aggravated by the prolonged use of sucrose.

These considerations seem sufficient to merit a more thorough investigation into the effect of replacing sucrose in the diet with levulose, not only for the diabetic, but for the normal person as well.

There is, then, a striking need for levulose, in order that the above suggestions may be subjected to more thorough experimental investigation.

The object of this paper is to present experimental data designed to improve upon the previously reported methods for the preparation of levulose and thus make it more quickly commercially available.

#### HISTORICAL

The history of the preparation and discovery of levulose, as well as its parent carbohydrate, inulin, has been summarized by Harding<sup>9</sup>. Since the date of this summary important work has been done by Hoche<sup>10</sup>, using chicory as the raw material, and by Jackson, Silsbee and Proffitt<sup>3</sup>, using Jerusalem artichokes and dahlias as raw materials. The latter workers revived the old Dubrunfaut<sup>11</sup> method of separating levulose by precipitation with lime, making improvements in the technique used in order to produce a more easily filterable precipitate. They also were able, as well as Hoche<sup>10</sup>, to crystallize levulose from aqueous solution without the use of organic solvents.

#### EXPERIMENTAL

The necessity for the immediate utilization of sugar beets has long been a handicap to the beet sugar industry. Since the storage of beets is impractical or entirely unsuccessful, the most natural solution to the problem would be to dry the beets. The tendency has been, however, to build the factories sufficiently large to dispose of the beets in season (usually about three months) and close down for the remainder of the year.

Only within recent years has the successful and profitable desiccation of sugar beets been accomplished. The problem, with its history and solution, is discussed by Owen<sup>12</sup>.

<sup>7</sup>Joslin, "Diabetic Metabolism with High and Low Diets," Carnegie Inst. Pub., 323:211 (1923).

<sup>8</sup>Statistical Abstracts of the United States, 42:79 (1919); *ibid.*, 48:75 (1925); *ibid.*, 49:77 (1926); *ibid.*, 50:82, 666 (1928); *ibid.*, 51:82 (1929).

<sup>9</sup>Harding, Sugar, 25:406, 636 (1923).

<sup>10</sup>Hoche, Z. Ver. deut. Zuckerind., 76:821 (1926); Sugar, 29:181 (1927).

<sup>11</sup>Dubrunfaut, Ann. Chim. phys., 21:169 (1847).

<sup>12</sup>Owen, "Desiccation of Sugar Beet," His Majesty's Stationery Office, London (1927).

This same problem is a natural fore-runner of the levulose industry. Only one reference has been found in the literature on the drying of Jerusalem artichokes. Nichols<sup>13</sup> described conditions for the drying of artichokes, evidently for the purpose of using them as food. No analytical data were given to show the effect of the drying on the constituents present. Since these data are essential if the artichokes are to be used for levulose production, the present study has been made.

THE EFFECT OF DRYING JERUSALEM ARTICHOKEs ON THE SUGAR CONTENT<sup>14</sup>

The tubers were ground through a food chopper, thoroughly mixed, 30 g. samples taken and either analyzed directly or dried and then analyzed. The data obtained are summarized in table 1. All sugar analyses in this paper were by Ost's copper carbonate method as modified by Nyns<sup>15</sup> and used by Oliver<sup>16</sup>, and Traub, Thor, Willaman and Oliver<sup>17</sup>. The percentages given are based on the 30 g. wet sample taken in every case. The percentage moisture in the wet sample was 77.45 per cent.

TABLE 1. *The analysis of fresh and dried Jerusalem artichokes*

No.	Sample	Fructose	Glucose	Total sugar	Fructose
					Total sugar
1.	Fresh tubers	11.95	2.85	14.80	0.807
2.	Dried at 100°C. 1 hr. <sup>18</sup>	12.04	1.99	14.03	0.858
3.	Dried at 90°C. 1 ¼ hr. <sup>18</sup>	12.12	2.43	14.55	0.833
4.	Dried at 80°C. 2 ¼ hr.	11.90	2.91	14.81	0.803
5.	Dried at 70°C. 2 ½ hr.	12.21	2.81	15.02	0.812
6.	Dried at 125°C. ¼ hr. then at 75°C. 1 hr.	11.88	2.98	14.86	0.799

THE EFFECT OF DRYING JERUSALEM ARTICHOKEs ON THE COAGULATION OF ALBUMINOUS MATTER

The moisture and total nitrogen were determined in the fresh tubers and in tubers dried at 75° to 80° C. for two hours and also for six hours. Samples of the fresh tubers and also the dried tubers were extracted with 100 cc. of hot water, using an extractor similar to the one described by Gardner and Kerone<sup>19</sup>, and the total nitrogen determined in the extracted pulp. In some cases the coagulable nitrogen (as precipitated by the Stutzer-Barnstein<sup>20</sup> method) was determined in the extract and in other cases the

<sup>13</sup>Nichols, Fruit Products J. and Am. Vinegar Ind., 9:71 (1929); Chem. Abstr., 24:440 (1930).

<sup>14</sup>The author is grateful to Dr. E. S. Haber of the Horticulture Department, Iowa State College, for furnishing the Jerusalem artichokes used throughout these experiments.

<sup>15</sup>Nyns, Bull. assoc. ecole sup. brasserie Louvain, 25:63 (1925); Chem. Abstr., 19:1236 (1925).

<sup>16</sup>Oliver, The Development of Methods for the Analysis of Inulin Bearing Plants. (1927.) (Unpublished Master's Thesis. Copy on file Univ. of Minn. Libr.)

<sup>17</sup>Traub, Thor, Willaman and Oliver, Plant Physiol., 4:123 (1929).

<sup>18</sup>These dried samples were dark brown in color, indicating caramelization.

<sup>19</sup>Gardner and Kerone, J. Chem. Education, 7:593 (1930).

<sup>20</sup>Barnstein, Landw. Vers. Stat., 54:327 (1900); Chem. Zentr., II, 1037 (1900).

total nitrogen in the extract was determined. All nitrogen determinations were made by the Kjeldahl method. The results are summarized in table 2. All percentages are based on the original sample weight.

TABLE 2. *Nitrogen content of the extract and the extracted pulp of Jerusalem artichokes*

Sample	Mois- ture	Nitrogen wet basis	Nitrogen dry basis	Pctg. of total N in pulp	Pctg. of total N in extr.
Fresh tubers	80.40	0.403	2.060		
Dried 75°-80° C. 2 hrs.	6.69	1.824	1.950		
Extracted pulp (a)		0.120	0.612	29.73	
Coagulable albumins (b)		0.138	0.704		34.16
Extracted pulp (c)		0.639	0.684	35.11	
Coagulable albumins (d)		0.651	0.697		35.76
Dried 75°-80° C. 6 hrs.	4.57	1.900	1.990		
Extracted pulp (e)		0.666	0.697	35.02	
Coagulable albumins (f)		0.597	0.625		31.40
Total extract (g)		1.184	1.240		62.31
Total extract (h)		0.283	1.443		70.04

(a) From fresh tubers. (b) In extract from fresh tubers. (c) From sample dried at 75°-80° C. for 2 hrs. (d) In extract from sample (c). (e) From sample dried at 75°-80° C. for 6 hrs. (f) In extract from sample (c). (g) From sample (c). (h) From fresh tubers.

#### THE EFFECT OF DRYING JERUALEM ARTICHOKE ON THE EASE OF DIFFUSION

Seven g. samples of dried artichoke chips (6.7 per cent water) were placed in a series of 150 cc. beakers, in a water bath at 80°-90° C. and extracted by adding 50 cc. of hot (90° C.) distilled water to beaker No. 1, allowed to heat for 10 minutes, the extract decanted into beaker No. 2 and fresh water added to No. 1, etc., thus simulating a diffusion battery. The refractive index was taken of the extract in each beaker at the end of every 10 min. period. The maximum value obtained was 1.4290 (28° C.), corresponding to 55 per cent total solids, after 17 samples had been added to the series. (The refractive index had not become constant, but was continuing to increase.) The first sample was completely exhausted after four extractions.

The same procedure, using an equivalent weight of fresh artichoke chips, gave a maximum value of 1.3588 (corresponding to 17.5 per cent total solids) which was constant after the eighth sample. The first sample was not completely exhausted until after eight extractions.

#### THE EFFECT OF DRYING JERUSALEM ARTICHOKE ON MAINTAINING THE LEVULOSE-GLUCOSE RATIO IN STORAGE

The results of the analysis of fresh and dried Jerusalem artichokes, before and after storage, are given in table 3.



TABLE 3. *The sugar content of Jerusalem artichokes before and after storage*

Sample	Mois- ture	Fruc- tose	Glu- cose	Total sugar	Fructose
					Total sugar
Freshly dug	77.45	11.94	2.85	14.79	0.807
Stored 5 months (a)	76.21	10.14	4.32	14.46	0.701
Freshly dug (dried)	6.10	34.51	7.85	42.36	0.814
Stored 5 months (b)	6.21	34.35	8.03	42.38	0.810

(a) Stored in root cellar for 3 months, then in stoppered bottle in refrigerator at 8° C. for 2 months.

(b) Dried at 75°-80° C. for 2¼ hrs. Stored in stoppered bottle.

#### THE EFFECT OF DRYING JERUSALEM ARTICHOKEs ON THE SOLUBILITY OF UNDESIRABLE CONSTITUENTS

The total extractable solids and ash (sulfated) were determined on a sample of fresh tubers. The extraction was made as for the nitrogen determinations above. The values found were: total solids 13.85 per cent; ash 1.13 per cent.

The determinations were repeated on the same sample after drying at 75°-80° C. for four hours. Percentages given are based on the original wet sample weight: total solids 13.05 per cent; ash 1.04 per cent.

#### THE PREPARATION OF LEVULOSE FROM JERUSALEM ARTICHOKEs

The method of the Bureau of Standards<sup>3</sup> was tried on both the fresh and dried artichokes. The method of clarification proved to be more effective on the extract from the dried tubers than from the fresh tubers. The lime levulate precipitation was satisfactory on small scale preparations but was found to be tedious and time consuming when applied to larger quantities of material. Greater success was attained by reverting to the old Dubrunfaut<sup>11</sup> method of simply adding cold slaked lime to the cold clarified juices. The lime levulate was then separated by centrifuging. The sirup obtained from dried artichokes crystallized in 24 hours.

#### CONCLUSION

Jerusalem artichokes can be dried without harming the carbohydrates present provided that the final temperature does not exceed 80° C. The chips should be white when dry. If they are caramelized the total sugar is found by analysis to have decreased, but the levulose to have remained unchanged. The temperature during the first stages of drying may be as high as 125° C. without injury to the chips.

Distinct advantages result from drying Jerusalem artichokes before extracting them for the production of levulose. Drying coagulates the albuminous matter and causes an increase of about five per cent of the total albuminoids to be retained by the pulp upon extraction. The drying also



renders the soluble albuminous matter more easily coagulable, and thus simplifies the clarification of the extract. Prolonged heating (after drying) at 75°-80° C. does not increase the amount of coagulation. The drying breaks down the cell structure and makes diffusion more easily accomplished. The levulose-total sugar ratio remains unchanged in dried tubers when stored. Thus smaller factories could be built and operated throughout the year. The drying could be done by local drying stations (removing both dirt and water) and the shipping costs materially reduced.

#### SUMMARY

The optimum temperatures for the drying of Jerusalem artichokes have been determined, and many advantages of drying the artichokes before the isolation of levulose have been proven.

# THE NUTRITIVE VALUE OF CEREALS AND ITS RELATION TO PROCESSING<sup>1</sup>

THOMAS B. PARKS

*From the Department of Chemistry, Iowa State College*

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A soft white variety of winter wheat used in the manufacture of Pettijohn's breakfast food, the scoured product, and Pettijohn's breakfast food were investigated to determine their content of the vitamin B complex. The term vitamin B complex refers to the mixture of vitamins B and G. The growth curves of rats were practically the same on diets containing comparative levels from eight to 20 per cent of the white wheat, scoured white wheat, and Pettijohn's breakfast food as the sole source of the vitamin B complex in the ration. Fifteen per cent of either white wheat, scoured white wheat, or Pettijohn's breakfast food incorporated in a diet, adequate in every respect other than the vitamin B complex, produces normal growth in the rat. These results show that there is no apparent destruction of the vitamin B complex in the process of manufacturing Pettijohn's breakfast food from white wheat. Reproduction was below normal on these diets, and most of the young produced, died within 48 hours after birth.

A study was made to determine the effect of processing on the vitamin B complex in the manufacture of puffed wheat from amber durum wheat. Growth was appreciably better on the cleaned durum wheat than on the pearled durum wheat and puffed wheat diets, when fed at comparative levels. Normal growth was obtained on the 14 per cent level of cleaned durum wheat, while at least 16 per cent of the pearled durum wheat was required to supply an adequate amount of the vitamin B complex for normal growth. The bran or scorings obtained in the pearling process is a relatively rich source of the vitamin B complex. This is shown by the fact that the growth curves were appreciably above the normal on diets containing 15 per cent of this material as the sole source of the vitamin B complex. As a result, the pearled product is not as rich a source of the vitamin B complex as the cleaned durum wheat. No appreciable growth was obtained on puffed wheat, even when it constituted 73.3 per cent of the diet as the sole source of the vitamin B complex. The high temperature and pressure to which the pearled puffing wheat is subjected in the puffing process destroys a considerable amount of the vitamin B complex—particularly the heat labile or antineuritic factor. Diets containing from 14 to 25 per cent of cleaned puffing wheat and pearled puffing wheat were adequate for the production of a large number of young, but were inadequate for lactation. No young were produced on the puffed wheat rations.

Whole oats and hulled oats are inferior to wheat as sources of the vitamin B complex. There appears to be but little difference in the content

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<sup>1</sup>An abstract of a thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

of the vitamin B complex in cleaned oats, dried oats, oat groats, and rolled oats. The growth curves of rats on diets containing 15 per cent levels of these substances as the sole source of the vitamin B complex were approximately nine per cent below the normal in each case. Growth was just below normal on 30 per cent levels of cleaned oats, dried oats, and oat groats. Oat hulls contain appreciable amounts of the vitamin B complex. A diet containing 40 per cent of oat hulls as the sole source of the vitamin B complex was adequate for considerable growth and for the production of young. The relatively poor growth on the oat diets was probably due to a deficiency of vitamin G, since Smith and Hendrick<sup>2</sup> obtained good growth upon the addition of autoclaved yeast to rolled oat diets.

A large percentage of the females on the different wheat diets, and to a much lesser degree on the various oat rations, died either during the period of pregnancy, at parturition, or shortly after giving birth to young. The mortality of the females at these periods was greater on the lower levels of the substances tested. This indicates that the vitamin B complex plays an important role in the well-being of the female during pregnancy and at parturition.

Rolled oats constitute a good source of protein. The nutritive value of rolled oat proteins for growth is approximately 84 per cent as compared with that of the proteins of whole milk powder; and they are superior to casein when fed at comparative levels of eight to 13.6 per cent of protein.

The method of Osborne, Mendel, and Ferry<sup>3</sup> was used in the comparative study of the protein values of rolled oats, of whole milk powder, and of casein. Between 8.5 and 9.6 per cent of oat proteins represent the minimum necessary for normal growth. Growth and reproduction were normal on 9.6 per cent of oat proteins. Rearing of young is not normal on this level however. On 10.02 per cent of rolled oat proteins, cooked and fed moist, growth, reproduction, and the number of young reared were normal; however, the young were slightly below normal weight at birth and at the weaning age.

Whole milk and rolled oats in certain proportions constitute a complete food. Forty per cent of rolled oats and 60 per cent of whole milk give normal growth and reproduction through the third generation. Rearing of young is slightly below normal on this ratio. Rolled oats and whole milk fed *ad libitum* give better than normal results for growth, reproduction, and lactation. The ratio of foods consumed was 28 per cent of rolled oats and 72 per cent of whole milk. Exceptionally vigorous animals were obtained on this mixture. Rolled oats and whole milk together with sugar and sodium chloride in the proportion consumed by the human being (rolled oats 46.03, milk 41.84, NaCl 2.37, cane sugar 9.76) constitute a complete food for growth, reproduction, and lactation. Normal growth and reproduction were obtained on mixtures of rolled oats, cream, sugar, and sodium chloride in the proportions used by the human being (rolled oats 46.03, cream, 41.84, cane sugar 9.76, NaCl 2.37). Lactation was slightly below normal on this diet.

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<sup>2</sup>M. I. Smith and E. G. Hendrick. U. S. Pub. Health Service Repts., 41:201 (1926).

<sup>3</sup>T. B. Osborne, L. B. Mendel and E. L. Ferry, J. Biol. Chem., 37:223 (1919).

# INSECTICIDAL AND CHEMICAL STUDIES OF PYRETHRUM AND KEROSENE EXTRACTS OF PYRETHRUM<sup>1</sup>

HENRY HOWE RICHARDSON

*From the Department of Zoology and Entomology, Iowa State College*

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Pyrethrum powder consists of the ground flower heads of the flower *Chrysanthemum cinerariaefolium* (Trev.) Boec. This flower contains two very active insect poisons, pyrethrins I and II (1). Two other species of *Chrysanthemum* are known to contain these active principles but *C. cinerariaefolium* is in most common use. The pyrethrins are soluble in most organic solvents (2, 3), and in recent years the use of kerosene extracts of pyrethrum has been taken up for the control of the house fly (*Musca domestica* L.) and other common household pests.

An insecticidal method for the evaluation of kerosene extracts of pyrethrum was developed first (4). Forty to sixty house flies of uniform age were loosed in a screen cage kept at constant temperature (83°F.) in an inclosure and sprayed with a constant amount of solution. The same atomizing apparatus was always used and atomization took place at a constant air pressure (5 lbs.). The spray was distributed in the cage by means of a fan which revolved at a constant speed.

When a kerosene extract of pyrethrum is applied in this way to flies, they are first stimulated to great activity; paralysis of the wings and legs follows and finally the fly falls on its back. This point is taken as the end point of paralysis and at thirty-second intervals, counts are made of the number of paralyzed flies. At the end of eight minutes the majority of the flies are paralyzed even with the weak pyrethrum extracts. The total number of flies used is recorded and the time necessary to paralyze 50 per cent of the flies is calculated by interpolation. After 24 hours, counts of the number of dead and live flies are made and the percentage of mortality obtained. It was found that the 50 per cent paralytic time, records very sensitively varying concentrations of pyrethrum extracts; the greater the concentration, the faster the speed of paralytic action. The mortality was found to vary only with greatly different concentrations of extract. For investigating the insecticidal power of pyrethrum extracts varying in concentration near the household spray strength (1 lb. of pyrethrum powder, 0.30 per cent pyrethrin I, to 1 gal. of kerosene), the 50 per cent paralytic time was without doubt the most useful and accurate criterion. Mathematical methods indicated that the differences in this time between three-fourths pound and one pound per gallon extracts were significant.

For the determination of the most efficient kerosene fraction for use in kerosene extracts of pyrethrum of the household spray type, a series of kerosenes (boiling range 310°-516° F.) were tested by the insecticidal method just described. It was found that the different fractions vary greatly in in-

<sup>1</sup>An abstract of a thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree Doctor of Philosophy.



secticidal power. A light fraction (48° Baumé, 310-395° F. boiling range) was decidedly slower in its speed of paralytic action and much less toxic in its final kill than a heavier fraction (41° Baumé, 378°-516° F. boiling range). Little difference was noted in a series of three short-cut fractions between 400° to 500° F. boiling range. Kerosenes refined with liquid sulfur dioxide (Edeleanu refining process) (5) showed little difference in toxicity from other fractions of the same boiling range.

In order to ascertain whether pyrethrum powders of varying degrees of fineness affect the extractive efficiency of kerosene percolation flowers from the same lot of previously well mixed material were ground to 15, 20, 30 and 45 mesh. A commercially ground 200-mesh powder was also tested. Comparative insecticidal tests were then made between the kerosene extracts of these powders and standard extracts<sup>1</sup>. There were no differences between extracts of powders varying from 15 to 45 mesh. Comparisons with standard extracts indicated 75 to 80 per cent extraction of the pyrethrum. About 90 per cent extraction was obtained with 200-mesh powder. There was then an increase in extractive efficiency with the very fine powder.

Tattersfield's short acid method (6, 7, 8, 9) for the analysis of pyrethrin I was found to give duplicate results. Insecticidal tests with extracts of several powders of different pyrethrin I content indicated quite clearly that the pyrethrin I content gave a reliable index of insecticidal power. With an artificially deteriorated pyrethrum powder (by exposure to alternate conditions of dampness and dryness) the chemical analysis was still found to give an accurate evaluation of insecticidal power.

To determine whether kerosene extracts of pyrethrum deteriorate with age, the differences in speeds of paralytic action between kerosene and the extract at the time the extract was prepared were compared with the differences obtained after the extract had aged for various periods of time; in these cases 8, 10 and 12 months. The extracts were kept in corked transparent-glass bottles and exposed to alternate conditions of darkness and light. The same type of control kerosene was used in all cases and it was assumed that its relative speed of paralytic action did not change. The experiments indicated that no loss of insecticidal power took place during these aging periods.

In studies with the experimental insect, tests with pyrethrum extract indicated that it had little repellent action toward house flies. Normally, with household spray strengths of pyrethrum extract, a considerable number of the flies recover after having been completely paralyzed. The fertility of these recovered flies was not affected; they laid fertile eggs which in turn developed into fertile adults.

A new technique of rearing flies was developed; this was especially suitable to the needs of the investigations. Experiments on the resistance of these flies at various ages (10, 11) to the toxic action of kerosene and kerosene extracts of pyrethrum indicated that the flies are most resistant (as judged by the final mortality) from 24 to 48 hours of age; after this

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<sup>1</sup>Such extracts were prepared by complete extraction of a known amount of pyrethrum with petroleum ether (20°-40° B. R.) in a Soxhlet extractor. The solvent was then evaporated with slight warming in a current of CO<sub>2</sub> and the residue taken up in a proportionate amount of kerosene to form a standard or known solution.



their resistance decreased. On the other hand, the flies' resistance to the paralytic action of pyrethrum extracts was least at the younger age; increased as they became older.

The resistance of any one lot of flies was usually quite uniform. However, the resistance of different lots of flies varied greatly from week to week and month to month. Over a period from January 15 to April 1, 1930, the mortality produced by kerosene on two-day old flies varied from a minimum of 9 per cent to a maximum of 81 per cent; the average mortality was 50.9 per cent.

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# REPRODUCTION AND LACTATION ON SIMPLIFIED DIETS<sup>1</sup>

MILTON WIGHT TAYLOR

*From the Department of Chemistry, Iowa State College*

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Evans and Bishop (1) and Sure (2) have stated that cod liver oil does not contain vitamin E. However, Nelson and associates (3, 4) obtained good reproduction through several generations of rats that were fed a diet in which cod liver oil (Squibb's) and yeast were the only sources of vitamins. In this paper is presented further evidence of the potency of cod liver oil (Squibb's) in vitamin E.

Reproduction was found to be normal on a diet of casein 18, salt mixture (No. 185) 3.7, cod liver oil (Squibb's) 5, ether extracted wheat germ 12, and dextrin. This was found to be true over long periods of time and through several generations. Rats on this diet remained fertile for as long as eighteen months. The fifth generation was obtained before it was decided to terminate the experiment. When butter fat was substituted for the cod liver oil in the aforementioned ration, very little reproduction occurred. This fact indicated that the extracted wheat germ did not contain vitamin E. Hence, the reproduction on the first mentioned diet must have been due to the presence of vitamin E in the cod liver oil.

The addition of petroleum ether or mineral oil to a diet containing wheat germ oil, a potent source of vitamin E, was found to prevent reproduction. Large amounts of petroleum ether (0.5 per cent of the diet) caused rats to exhibit signs of vitamin A deficiency. On diets containing only a trace of petroleum ether, growth was normal, but reproduction was not. Lack of reproduction may have been due to the prevention of absorption of vitamin A and possibly of vitamin E.

One sample of crude cane molasses was found to be a good source of vitamin E. It was fed at three and five per cent levels in rations whose only other sources of vitamins were five per cent of butter fat and twelve per cent of yeast. The animals on these diets remained fertile for eleven months. Three kinds of beet molasses and sorghum were found to contain some vitamin E, though not as much as the first sample of crude cane molasses. A second sample of crude cane molasses, refined cane molasses, honey, the ash of wheat germ oil, and the ash of crude cane molasses did not maintain fertility. Although dried cane molasses and the fat from this dried molasses gave no reproduction, the dried and fat-free molasses appeared to be a good source of vitamin E. At least, eight females, five of which died near the beginning of the experiment, produced thirteen litters of young in thirteen months on the diet.

The great lack of reproduction on several different diets with large numbers of animals led to an investigation of the fertility of the two sexes by means of matings with normal animals from the stock colony. All of

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<sup>1</sup>An abstract of a thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

the males, thirty-four in number, which had been fed different synthetic diets for a period of about seven months were found to be sterile. Eighty-three females, which had been on the same diets for the same time, were placed with normal males from the stock colony for periods varying from three to ten months. About eighty-five per cent of these females were still able to produce young. In fact, most of them remained fertile for a long period of time and produced several litters each. It was concluded from these data that either the male rat requires a much larger amount of vitamin E than does the female or that the male requires some factor other than vitamin E which the female does not require. It is believed that a chance fertility of one male may account for the good reproduction obtained on certain diets on which reproduction was not expected.

Waddell and Steenbock (5) have reported that one per cent of ferric chloride in a ration will destroy the vitamin E completely, but will not affect the vitamin A. Results obtained in this laboratory do not confirm this statement. Growth on diets containing ferric chloride was much poorer than was the case on the same diets which contained no ferric chloride. Many of the rats died, showing decided symptoms of vitamin A deficiency. The effect did not seem to be caused by the ingestion of ferric chloride, since growth was very poor on diets containing cod liver oil or wheat germ oil which had been treated with ferric chloride in much the same way as was done in the ration, and from which the ferric chloride had been removed by washing with water. These effects are believed to be caused by the formation of peroxides or oxidative catalysts which subsequently destroy the vitamin A in the ration. It was found that the testes of male rats fed diets containing ferric chloride showed some degeneration. However, this degeneration was not nearly as complete as in the case of rats fed diets high in fat and deficient in vitamin E.

Several investigators have suggested that some dietary factor is necessary for lactation. Sure (6) stated that wheat germ oil contained both this factor and vitamin E. The results obtained in this laboratory do not confirm Sure's statement. The addition of wheat germ oil to a diet of casein, salts, butter fat, yeast and dextrin did cause a noticeable increase in the number of young weaned. However, on a diet of casein, salts, cod liver oil, extracted wheat germ and dextrin, the addition of wheat germ oil gave much poorer results on lactation. Lactation on diets containing crude cane molasses was very good.

Grains have been shown to be good sources of vitamin B, but not of vitamin G. Lactation was studied on diets containing white corn, yellow corn, barley, hulled oats, rye and wheat as sources of vitamins B and G. Poor results were obtained on all the diets except the one containing hulled oats. Supplementing these diets with ten per cent of yeast or of autoclaved yeast gave very good lactation. It was evident that vitamin G was the limiting factor in lactation on these diets.

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